JEFFREY KEITH TOMBERLIN
Biological, Behavioral, and Toxicological Studies on the Black Soldier Fly (Diptera: Stratiomyidae)
(Under the Direction of: RAY NOBLET)

Black soldier flies (BSF), *Hermetia illucens* (L.), were reared on three media to determine effects of diet on pre-imaginal development and selected adult life-history traits. Characteristics examined for individuals reared on each diet were compared to field-collected prepupae and corresponding adults. Diet-reared specimens had reduced emergence, size, longevity, and calorie content in comparison to wild specimens.

Time from emergence to mating and oviposition for colony-reared BSF placed in a cage in a greenhouse was examined. Sixty-nine percent of mating occurred 2 d after eclosion and 70% of oviposition 2 d after mating. Mating was significantly correlated with time of day and light intensity, while oviposition significantly correlated with time of day, temperature, and humidity.

Oviposition site selection was examined for BSF in a greenhouse. Adults oviposited in two treatments: Gainesville house fly (HF), *Musca domestica* larval diet (1) with and (2) without BSF larvae. Number of egg clutches collected per treatment did not differ.

Cyromazine and pyriproxifen LC_{50}s for BSF larvae failing to become adults ranged from 0.13 - 0.19 ppm and 0.10 to 0.12 ppm respectively. Slopes of dosage-mortality regression for BSF larvae fed larval media treated with cyromazine or pyriproxifen ranged from 3.94 - 7.69 and 1.67 - 2.32 respectively. Pyriproxifen dosage-mortality regressions were not generated for percent larvae failing to become prepupae

since < 32% mortality occurred at any rate. Pyriproxifen rates from 0.03 to 232 ppm resulted in 12 - 29% greater prepupal weights than the control.

Dosage-mortality regressions were determined for BSF, Cornell-susceptible HF and wild HF exposed to two pyrethroids: λ-cyhalothrin, and permethrin. The BSF population is much more sensitive to these pyrethroids in comparison to the Cornell-colony and wild HF population.

Olfactory and tactile inhibition of HF oviposition in media inoculated with BSF larvae was investigated. Results from the oviposition bioassays suggest that olfactory cues inhibit HF oviposition in media inoculated with BSF larvae. However, results from the olfactometer experiments were inconclusive and additional research is needed to more clearly understand these interactions.

INDEX WORDS: Entomology, Black Soldier Fly, Toxicology, Biology, Behavior

BIOLOGICAL, BEHAVIORAL, AND TOXICOLOGICAL STUDIES ON THE BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE)

by

JEFFERY KEITH TOMBERLIN

B.S., The University of Georgia, 1993

M.S., Clemson University, 1996

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2001

BIOLOGICAL, BEHAVIORAL, AND TOXICOLOGICAL STUDIES ON THE BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE)

by

JEFFERY KEITH TOMBERLIN

Approved:

Major Professor: Ray Noblet

Committee: John Ruberson
Craig Sheppard
Wayne Berisford
Brian Forschler

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
August 2001

ACKNOWLEDGMENTS

First and foremost I would like to thank my Lord and Savior Jesus Christ for giving me the strength and fortitude to complete this degree. I would like to especially thank Dr. Craig Sheppard for not only serving as my advisor, but for being a great friend. Thanks are also extended to my committee members for their assistance during the completion of my Ph.D. Additionally, thanks are extended to Dr. Jerry Butler for not only serving on my committee but for allowing me to work in his laboratory and use his olfactometer.

I would like to express my gratitude to John Joyce, Barbara Kiser, Sonya Sumner, Richard Layton, the staff in the entomology and information technology offices for their assistance in completing my degree. I also would like to thank the Rogers family for their support during the past two years. They provided encouraging words during some of my most difficult periods as a graduate student.

In closing I would like to thank my family. Each member of my family has been there when I was in greatest need. I love each of you with all of my heart and I will always be grateful for your support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Georgia Livestock Industry

In 1999, poultry represented 48% of Georgia's agricultural production and was valued at 2.7 billion dollars (Abbe and Messer 2000). The demand for poultry goods has resulted in record levels of production and sales (Abbe and Messer 2000) with new facilities being built each year in order to increase statewide production. However, increased facilities also result in an increase in poultry by-products, specifically wastes. Any increase in waste production is viewed negatively due to possible environmental threats, such as a reduction in air and water quality.

Environmental and Political Concerns

Wastes from livestock and poultry can have negative effects on ground and surface waters (Hoban et al. 1997). In the past, waste was disposed of through various practices, such as anaerobic digestion in lagoons prior to being applied as fertilizer on fields (Mallin et al. 1997). However, these methods could potentially pollute surrounding ground and surface waters if not managed properly (Chapman 1996). Current waste management practices in poultry facilities rely on keeping the manure dry (Fatchurochim et al. 1989) and removing it from the facilities once a year (Axtell and Arends 1990).

Many of the current waste-management practices used by livestock facilities are expected to become obsolete due to modifications of federal and state regulations intended to increase environmental protection (Morse 1995 and 1996). For example, regulations are being developed to limit the amount of manure applied to fields as fertilizer. Therefore, alternative methods, which are more environmentally sound and easily implemented, need to be developed and made accessible to the livestock industry (Morse 1996).

House Fly, *Musca domestica* L.: A Major People Pest

The house fly, *Musca domestica*, is considered a major pest of man (Axtell 1986, Van Horn 1995). House flies are common in livestock facilities, such as poultry houses, and immatures develop in the manure (Greenberg 1971). Flies may disperse from poultry facilities and enter homes and businesses where they are viewed as a nuisance (Axtell 1970). Also, the house fly is known to transmit livestock and human pathogens (Williams et al. 1985, Iwasa et al. 1999).

Managing house fly populations is a paramount concern to the livestock industry. The U.S. poultry industry spends an estimated 40 million dollars annually on house fly control (Axtell 1986). Egg producers alone spend an estimated 13 cents per bird per year, or $13,000 per facility, on house fly control (Axtell 1986). Research programs are continuously searching for less expensive alternatives for controlling house flies that do not compromise environmental integrity (Axtell and Arends 1990).

Cultural Control of Arthropod Pests in Poultry Facilities

Cultural control is the manipulation of an environment to reduce a pest population below its economic threshold (Pedigo 1989). Examples of cultural control in agriculture include sanitation, modification of pest habitat, tillage, and water management (Pedigo 1989). Procedures, such as those listed, are part of pest control programs used by the livestock industry today (Axtell and Arends 1990, Van Driesche and Bellows Jr. 1996).

Facility Temperature and Relative Humidity

Temperature and relative humidity can be manipulated for controlling various pest arthropods within livestock facilities (Stafford and Collison 1987, Lysyk 1998). House flies and many other poultry pests, such as the lesser mealworm, *Alphitobius diaperinus*

(Panzer), and the hide beetle, *Dermestes maculatus* Degeer, are sensitive to these factors (Stafford and Collison 1987, Fletcher et al. 1990). Lower temperature and humidity within a livestock facility can slow the growth of pest populations, thereby limiting pest problems such as wood boring, or irritation of livestock workers (Stafford and Collison 1987). Accordingly, producers save money through a reduction in pest damage and control costs (Pedigo 1989).

Moisture Content of Manure

The level of moisture in manure affects dipteran populations (Axtell 1969, Greenberg 1971). Fatchurochim et al. (1989) determined that oviposition-site selection, by certain muscid fly pests of livestock (house fly, *Musca domestica* L.; black garbage fly, *Ophyra aenescens* (Wiedemann); false stable fly, *Musca stabulans* (Fallen); and the little house fly, *Fannia femoralis* (Stein)), is influenced significantly by moisture levels in manure. They determined that these species oviposit primarily in manure with moisture levels between 40-80%. Presently, integrated pest management (IPM) practices for poultry are designed to lower the manure moisture to 40% (Fatchurochim et al. 1989) through increased ventilation within the facility (Mullen et al. 1996a). At this moisture level the manure remains suitable for sustaining populations of beneficial insects while reducing pest species numbers (Stafford and Collison 1987).

Manure removal

Scheduled manure removals are an important part of IPM in livestock (Peck and Anderson 1970, Axtell 1986). Timing and frequency of the clean-outs greatly affect the rates at which arthropods (beneficial and pestiferous) multiply and colonize manure (Peck and Anderson 1970). As mentioned previously, manure that is allowed to accumulate and

remain moist is an excellent medium for the development of livestock pests (Anderson 1965, Axtell 1969, Greenberg 1971).

Legner et al. (1973) determined that manure allowed to accumulate often results in a more stable environment and decreased pest-fly density. They attributed this reduction in pest-fly density to increased time intervals between manure removals, which provide bio-control agents the necessary time to build up populations capable of suppressing pests. However, manure removal is inevitable (Mullens et al. 1996a) and must be properly scheduled in order to maintain the populations of biological control agents. Current trends for manure removal range from twice annually to once every two years, or partial removal annually (Mullens et al. 1996a). Partial removal allows some biological control agents to remain in the house and suppress pest species as they colonize the accumulating manure (Mullens et al. 1996b).

Biological Control of Pests in Poultry Facilities

Biological control is the use of any biological agent for the reduction of a pest species below its economic threshold (Huffaker and Messenger 1976, Pedigo 1989). Presently, several biological control agents are available for the livestock industry (Williams et al. 1985). These agents range from predaceous mites to pathogens (Axtell 1969, Legner et al. 1975, Miller et al. 1983, Geden 1990, Wills et al. 1990) and competitors (Furman et al. 1959, Sheppard 1983).

Pathogens

Approximately 1500 pathogens are known to attack arthropods (Miller et al. 1983). Of these, 30% are fungi, and two important species, *Entomophthora muscae* (Cohn) and *Beauveria bassiana* (Balsamo), have been identified as potential biological

control agents of fly pests associated with livestock (Hall and Papierok 1982). Both fungi are known to exist in natural populations of dipteran pests of livestock. (Mullens 1989, Watson and Petersen 1993). They are able to withstand harsh environmental conditions and easily infect hosts. Once within an individual, they can be transmitted horizontally through a fly population by individual contact. Additionally, a fungal infection reduces the number of offspring produced per female fly (Moller 1993). Techniques have been developed for their formulation and application (Kramer and Steinkraus 1987, Geden et al. 1993, Watson et al. 1996). However, there is little practical information on how to integrate their use into livestock IPM (Mullens 1986, Watson et al. 1996).

Competitive Exclusion

In the classical sense, biological control is achieved through the importation and release of an agent to suppress a pest population (Moon 1980a). Typically, the agent used to suppress the pest population is a predator, parasitoid, pathogen, or a combination of the three. However, biological control is possible without agents directly killing the pests. Moon (1980a) suggested that some pests can be suppressed by applying the principle of competitive exclusion.

Competitive exclusion is the suppression of a pest population through the use of a non-pest species to compete for resources essential for growth and development of the pest (Blume et al. 1973, Moon 1980b). Once a habitat is colonized by the non-pest species it becomes less suitable for the growth and development of the pest species (Blume et al. 1973, Moon 1980b). The black soldier fly, *Hermetia illucens* (L.), is a native species thought to be a competitive excluder of house flies in poultry facilities (Moon 1980a). Bradley and Sheppard (1984) suggested the black soldier fly not only

reduces larval house fly survival through competition for resources, but also releases allelochemicals that are repellent to ovipositing house flies.

Black Soldier Fly, *Hermetia illucens*, (Diptera: Stratiomyidae)

The black soldier fly is distinguished from other stratiomyid species by its large size (13 to 20 mm) and piebald pattern on its body (May 1961). It is distributed throughout the sub-tropical and tropical regions of the world (Furman et al. 1959, McCallan 1974) and is often found colonizing decomposing material, such as fruits, carrion (Dunn 1916, James 1935 and 1947), and poultry manure (Furman et al. 1959, Leclerq 1969, Axtell and Edwards 1975). In the southeastern United States, *H. illucens* has three generations annually (Sheppard et al. 1994) and can be collected from late spring through summer (Furman et al. 1959).

Egg clutches of the black soldier fly average 998 eggs per clutch and hatch after 102-105 hr at 24 ^{o}C (Booth and Sheppard 1984). Larvae fed a standard house fly medium passed through 6 larval stages over 31 d at 27.8 ^{o}C (May 1961). However, larval and pupal development can vary depending on environmental conditions (May 1961). Adults were thought to live approximately 4 d, and females lay one clutch of eggs per female (D.C.S. unpublished).

Biological control of house fly populations

In poultry, the black soldier fly is often thought to be a pest species because the larvae liquify manure, making it difficult to remove, as well as destabilize the foundation of the poultry facility (Axtell 1970, Axtell and Arends 1990). However, these reports are anecdotal and research on this subject shows that black soldier fly larvae dry manure that they occupy (Sheppard 1983). Fatchurochim et al. (1989) reported that black soldier fly

larvae grow best in manure with < 60% moisture, which is below the optimal moisture level for rearing house fly larvae.

The presence of soldier flies within a poultry facility may reduce manure accumulation and help to control house flies (Furman et al. 1959, Hale 1973, Sheppard 1983, Sheppard et al. 1994). Sheppard (1983) determined that soldier fly colonization of poultry manure reduced house fly production from 94 to 100%. Bradley and Sheppard (1984) determined that female house flies laid fewer eggs in manure already colonized by soldier flies. They suggested that nutrient depletion is the probable reason why house flies are unable to develop in manure colonized by soldier fly larvae.

No other method for house fly control offers more potential benefits than soldier flies. Cyromazine (Larvadex®, Norvartis, Greensboro, North Carolina) approximates the same level of control provided by the soldier fly (Sheppard 1983). However, house fly resistance to cyromazine is widespread (Sheppard et al. 1989). In contrast, there are no records of house flies being able to successfully compete with soldier fly larvae for nutrients in manure.

Conversion of waste to feed

Hermetia illucens reduces manure (Tingle et al. 1975) and the prepupae are a high quality feed for swine (Newton et al. 1977), fish (Bondari and Sheppard 1981), and poultry (Hale 1973). The concept of using manure as media to produce high quality feed is not new. Researchers have proposed using animal manure as a substrate on which maggots can be reared for feedstuffs (Miller 1969, Morgan and Eby 1975, Booram et al. 1977, Anderson 1980, Chiou and Chen 1982).

Nutritional analysis of soldier fly prepupae has shown them to be similar to soybean meal, a common feed for livestock (Booram et al. 1977). Sheppard et al. (1994) determined that an estimated 0.6 kg of *H. illucens* prepupae per hen might be produced annually in caged-layer houses inoculated with soldier flies. Larvae feed on the chicken manure and convert it to 42% protein and 35% fat (Newton et al. 1977). Swine preferred the prepupae over a soy based feed (Newton et al. 1977) and growth and development of the swine did not differ significantly between the two diets.

Methods used to harvest black soldier fly pre-pupae

An obstacle to large scale production of soldier fly prepupae as a feedstuff has been the lack of a method for harvesting the prepupae. Sheppard et al. (1994) reported a system for self-harvesting prepupae in a 460 hen caged layer house. The system used a modified house foundation, which had a 30 cm deep, 109 cm wide concrete manure basin under the cage batteries. The floor of the basin had an incline gradient of 40^{o} on the outer wall, while the central basin wall was 90^{o} (vertical). A 10 cm diameter plastic pipe with a 1.5 cm gap was attached along the top of the outer wall slope with the gap tightly fitted facing the incline. Prepupae would climb the incline and fall into the pipe. Once in the pipe, the prepupae traveled to the end of the pipe and fell into a collection center. The collection system was designed so that the current manure removal system would need no modifications. This design can also be expanded to fit state-of-the-art facilities.

Monetary rewards

Utilizing the black soldier fly as a biological control agent of house flies could produce large monetary returns for poultry producers. Larvadex® can cost as much as $0.10 per hen annually. Manure removal and surface application costs $0.28 per hen per

yr in high-rise houses. Soldier fly activity in poultry manure results in a 25% reduction in waste accumulated annually (Sheppard 1983). Using these waste reduction numbers, soldier flies could save a grower $0.07 per hen per year (0.25 x $0.28). Additional income could be acquired through the sale of harvested prepupae, which have an estimated value equal to soybean meal, which has a market price of $167-200 per ton (Anonymous 2001).

Conclusion

Manure management with black soldier flies offers many advantages: waste will be reduced by 25% annually, a high quality feedstuff will be produced, and house fly populations will be controlled. But most modern swine and poultry facilities exclude ovipositing black soldier flies. Mass colonization techniques are needed for reliable processing of the manure by the soldier fly larvae. Optimum utilization of this valuable system in commercial food animal production operations requires a better understanding of black soldier fly biology, and its interaction with currently used pesticides, as well as, its interactions with pest species, such as the house fly. My research investigated the: 1) general biology of the black soldier fly and improved rearing methods for this species, 2) susceptibility of soldier fly adult and larvae to various pesticides used in poultry facilities and, 3) intra- and interspecific olfactory interactions between the black soldier fly and the house fly.

References Cited

Abbe, D. S. and C. S. Messer. 2000. Georgia Agricultural Facts. Georgia Agricultural Statistics Service. Athens, Georiga. 99pp.

Anderson, J. R. 1965. A preliminary study of integrated fly control on northern California

poultry ranches. Proc. California Mosquito Control Assoc. 33: 43-44.

Anderson. W. 1980. Some insects. Feedstuffs 51: 22.

Anonymous. 2001. Market prices of agricultural commodities. Feedstuff 73: 24.

Axtell, R. C. 1969. Macrochelidae (Acarina: Mesostigmata) as biological control agents for synanthropic flies. Pages 401-416. In Proc. 2nd Int. Congr. Acarol., 1967. Akademai Kiado, Budapest.

Axtell, R. C. 1970. Integrated fly-control program for caged-poultry houses. J. Econ. Ent. 63: 400-405.

Axtell, R. C. 1986. Fly management in poultry production: Cultural, biological, and chemical. Poultry Sci. 65: 657-667.

Axtell, R. C. and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annu. Rev. Entomol. 35: 101-126.

Axtell, R. C. and T. D. Edwards. 1975. *Hermetia illucens* control in poultry manure by larvaciding. J. Econ. Entomol. 63: 1786-1787.

Blume, R. R., J. J. Matter, and J. L. Eschle. 1973. *Onthophagus gazella*: Effect on survival of horn flies in the laboratory. Environ. Entomol. 2: 811-813.

Bondari, K. and D. C. Sheppard. 1981. Soldier fly larvae as feed in commercial fish production. Aquac. 24: 103-109.

Booram, Jr., C. V., G. L. Newton, and R. W. Barker. 1977. Manure residue as a substrate for protein production via *Hermetia illucens* larvae. Proc. Cornell Agricultural Waste Management Conf.

Booth, D. C. and D. C. Sheppard. 1984. Oviposition of the black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae): Eggs, masses, timing, and site characteristics. Environ. Entomol. 13: 421-423.

Bradley, S. W. and D. C. Sheppard. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. J. Chem. Ecol. 10: 853-859.

Chapman, S. L. 1996. Soil and solid poultry waste nutrient management and water quality. Poultry Sci. 75: 862-866.

Chiou, Y. Y. and W. J. Chen. 1982. Production of the maggot protein reared with swine manure. Natl. Sci. Council Mo. (ROC) 10: 677-672.

Dunn, L. H. 1916. *Hermetia illucens* breeding in a human cadaver. Entomol. News 27: 59-61.

Fatchurochim, S., C. J. Geden, and R. C. Axtell. 1989. Filth fly (Diptera) oviposition and larval development in poultry manure of various moisture levels. J. Entomol. Sci. 24: 224-231.

Fletcher, M. G., R. C. Axtell, and R. E. Stinner. 1990. Longevity and fecundity of *Musca domestica* (Diptera: Muscidae) as a function of temperature. J. Med. Entomol. 27: 922-926.

Furman, D. P., R. D. Young, and E. P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52: 917-921.

Geden, C. J. 1990. Coleopteran and acarine predators of house fly immatures in poultry production systems, pp. 177-200. In Rutz, D.A. and R.S. Patterson (eds.), Biocontrol of Arthropod Pests of Livestock and Poultry. Westview, Boulder, Co.

Geden, C. J., D. C. Steinkraus, and D. A. Rutz. 1993. Evaluation of two methods for release of *Entomopthora muscae* (Entomophthorales: Entomopthoraceae) to infect house flies (Diptera: Muscidae) on dairy farms. Environ. Entomol. 20: 1201-1208.

Greenberg, B. 1971. Flies and Disease. Vol. 1. Ecology, Classification and Biotic Associations. Princeton Univ. Press, Princeton, NJ. 856 pp.

Hale, O. M. 1973. Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. J. Georgia Entomol. 8: 16-20.

Hall, R. A. and B. Papierok. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology 84: 205-240.

Hoban, T. J., W. B. Clifford, M. Futreal, and M. McMillan. 1997. North Carolina producers' adoption of waste management practices. J. Soil. and Water Conserv. 52: 332-339.

Huffaker, C. B., and P. S. Messenger. 1976. Theory and Practice of Biological Control. Academic Press, New York, New York. 743 pp.

Iwasa, M., S. Makino, H. Asakura, H. Kobori, and Y. Morimoto. 1999. Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol. 36: 108-112.

James, M. T. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bull. Brooklyn Entomol. Soc. 30: 165-170.

James, M. T. 1947. The flies that cause myiasis in man. Misc. Publ. US Dept. Agric. 631: 146-148.

Kramer, J. P. and D. C. Steinkraus. 1987. Experimental induction of the mycosis caused by *Entomopthora muscae* in a population of house flies (*Musca domestica*) within a poultry building. J. New York Entomol. Soc. 95: 114-117.

Leclerq, M. 1969. Dispersion et transport des insectes nuisibles: *Hermetia illucens* L. (Diptera: Stratiomyidae). Bull. Rech. Agron. Gembloux N.S. 4: 139-143.

Legner, E. F., W. R. Bowen, W. D. McKeen, W. F. Rooney, and R. F. Hobza. 1973. Inverse relationship between mass of breeding habitat and synanthropic fly emergence and the measurement of population densities with sticky tapes in California inland valleys. Environ. Entomol. 2: 199-205.

Legner, E. F., G. S. Olton, R. E. Eastwood, and E. J. Dietrick. 1975. Seasonal density, distribution and interactions of predatory and scavenger arthropods in accumulating poultry wastes in coastal and interior southern California. Entomophaga 20: 269-283.

Lysyk, T. J. 1998. Relationship between temperature and life-history parameters of *Stomoxys calcitrans* (Diptera: Muscidae). J. Med. Entomol. 35: 107-119.

Mallin, M. A., J. M. Burkholder, M. R. McIver, G. C. Shank, H. B. Glasgow, Jr., B. W. Touchette, and J. Springer. 1997. Comparative effects of poultry and swine waste lagoon spills on the quality of receiving streamwaters. J. Environ. Qual. 26: 1622-1631.

May, B. M. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). N.Z. J. Sci. 4: 55-65.

McCallan, E. 1974. *Hermetia illucens* (L.) (Diptera: Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. Entomol. Monthly Magazine 109: 232-234.

Miller, B. F. 1969. Biological digestion of manure by Diptera. Feedstuffs 51: 32.

Miller, L. K., A. J. Lingg, and L. A. Bulla, Jr. 1983. Bacterial, viral, and fungal insecticides. Science 219: 715-721.

Moller, A. P. 1993. A fungus infecting domestic flies manipulates sexual behaviour of its

host. Behav. Ecol. Sociobiol. 33: 403-407.

Moon, R. D. 1980a. Biological control through interspecific competition. Environ. Entomol. 9: 723-728.

Moon, R. D. 1980b. Effects of larval competition on face fly. Environ. Entomol. 9: 325-330.

Morgan, N. O. and H. J. Eby. 1975. Fly protein production from mechanically mixed animal wastes. Israel J. Entomol. 10: 73-81.

Morse, D. 1995. Environmental consideration of livestock producers. J. Anim. Sci. 73: 2733-2740.

Morse, D. 1996. Impact of water and air quality legislation on the poultry industry. Poultry Sci. 75: 857-861.

Mullens, B. A. 1986. A method for infecting large numbers of *Musca domestica* (Diptera: Muscidae) with *Entomopthora muscae* (Entomophthorales: Entomophthoraceae). J. Med. Entomol. 23: 457-458.

Mullens, B. A. 1989. Cross-transmission of *Entomopthora muscae* (Zygomycetes: Entomopthoraceae) among naturally infected muscoid fly (Diptera: Muscidae) hosts. J. Invert. Pathol. 53: 272-275.

Mullens, B. A., N. C. Hinkle, and C. E. Szijj. 1996a. Role of the poultry manure pad in manure drying and its potential relationship to filth fly control. J. Agric. Entomol. 13: 331-337.

Mullens, B. A., N. C. Hinkle, and C. E. Szijj. 1996b. Impact of alternating manure removal schedules on pest flies (Diptera: Muscidae) and associated predators (Coleoptera: Histeridae, Staphylinidae; Acarina: Macrochelidae) in caged-layer poultry manure in southern California. J. Econ. Entomol. 89: 1406-1417.

Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44: 395-400.

Peck, J. H. and J. R. Anderson. 1970. Influence of poultry-manure-removal schedules On various Diptera larvae and selected arthropod predators. J. Econ. Entomol. 63: 82-90.

Pedigo, L. P. 1989. Entomology and Pest Management. Macmillan Publishing Attention: Company. New York, NY. 646 pp.

Sheppard, D. C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1442.

Sheppard, D. C., N. C. Hinkle, J. S. Hunter III, and D. M. Gaydon. 1989. Resistance on constant exposure livestock control systems: a partial review with some original findings on cyromazine resistance in house flies. Fla. Entomol. 72: 360-369.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value added manure management system using the black soldier fly. Bio. Tech. 50: 275-279.

Stafford III, K. C. and C. H. Collison. 1987. Manure pit temperature and relative humidity of Pennsylvania high-rise poultry houses and their relationship to arthropod population development. Poultry Sci. 66: 1603-1611.

Steelman, C. D. and A. R. Colmer. 1970. Some effects of organic waste on aquatic insects in impounded habitats. Ann. Entomol. Soc. Amer. 63: 397-400.

Tingle, F. C., E. R. Mitchell, and W. W. Copeland. 1975. The soldier fly, *Hermetia illucens*, in poultry houses in north central Florida. J. Ga. Entomol. 10: 179-183.

Van Driesche, R. G. and T. S. Bellows, Jr. 1996. Biological Control. Chapman and Hall. New York, New York. 539 pp.

Van Horn, J. 1995. Proceedings: Nuisance concerns in animal manure management: Odors and flies. Florida Cooperative Extension, University of Florida. Gainesville, FL. 144 pp.

Watson, D. W. and J. J. Petersen. 1993. Sexual activity of male *Musca domestica* (Diptera: Muscidae) infected with *Entomopthora muscae* (Entomopthoraceae: Entomophthorales). Biological Control 3: 22-26.

Watson, D. W., D. A. Rutz, and S. J. Long. 1996. *Beauveria bassiana* and sawdust bedding for the management of the house fly, *Musca domestica* (Diptera: Muscidae) in calf hutches. Biological Control 7: 221-227.

Williams, R. E., R. D. Hall, A. B. Broce, P. J. Scholl. 1985. Livestock Entomology. John Wiley and Son, New York, New York. 335 pp.

Wills, L. E., B. A. Mullens, and J. D. Mandeville. 1990. Effects of pesticides on filth fly predators (Coleoptera: Histeridae, Staphylinidae; Acarina: Macrochelidae, Uropodidae) in caged-layer poultry manure. J. Econ. Entomol. 83: 451-457.

CHAPTER 2

SELECTED LIFE-HISTORY TRAITS OF BLACK SOLDIER FLIES (DIPTERA: STRATIOMYIDAE) REARED ON THREE ARTIFICIAL DIETS

Jeffery K. Tomberlin, D. Craig Sheppard and John A. Joyce. To be submitted to the Journal of Economic Entomology

Abstract

Hermetia illucens (L.) larvae were reared on a CSMA diet for house fly, *Musca domestica* L., larvae, Gainesville house fly larval diet, and a standard laying hen ration to determine the effects of diet on pre-imaginal development and selected adult life-history traits. Prepupal and adult characteristics examined for individuals reared on each diet were compared to field-collected prepupae and corresponding emergent adults. Diet did not significantly influence development or survivorship to the prepupal stage. However, adult emergence for all of the diet treatments was significantly less than that determined for the wild population. Development time from egg to adult for individuals reared on the diet treatments ranged from 40 to 43 d with the larval stage lasting 22 to 24 d at 27 ^{o}C. We observed > 95% larval survivorship to the prepupal stage and 21 to 27% adult emergence. For emerging adults, sex ratio did not significantly differ across treatments including the wild population. Percent diet residue of the dry matter fed to the larvae ranged from 35 to 37%, while final percent moisture of the residue ranged from 36 to 56%.

Specimens reared on each diet were reduced in size, longevity and calorie content in comparison to specimens from the wild population. Males within diet treatments and for field-collected specimens were significantly smaller than females and emerged 1 to 2 d prior to females. Additionally, males reared on the diet treatments lived for approximately 10 d, while females lived for approximately 8 d, when provided water. Females accounted for 55 to 60 % of emerging adults across treatments including the wild population sampled.

This information suggests that these diets may be used for rearing soldier flies in the lab. Selection of a suitable diet for mass rearing may be based on cost and availability of resources. However, further refinement is needed in order to produce adults similar to those found in nature.

Introduction

THE BLACK SOLDIER FLY, *Hermetia illucens* (L.), is distributed worldwide throughout the tropical and subtropical regions, including the southeastern United States (James 1935) where it is active from April to November (Sheppard et al. 1994). Despite its wide distribution, biological information on this species is limited (James 1935, May 1961, Booth and Sheppard 1984, Sheppard et al. 1994, Kemppinen 1998). More recent research has been concerned with benefits associated with soldier fly colonization of livestock and poultry manure, which include reducing manure accumulation, using the prepupae as livestock feed (Sheppard et al. 1994), and suppression of house fly, *Musca domestica* L., populations (Furman et al. 1959, Sheppard 1983, Bradley and Sheppard 1984),

Rearing of the black soldier fly has been attempted in the past (Tingle et al. 1975) but was unsuccessful until recently (D.C.S., unpublished data). With the development of suitable methods, the soldier fly can now be produced and made available as a biological control and waste management agent.

The primary objective of this study was to determine basic biological parameters for the black soldier fly in relation to various rearing media. Additionally, we compared life-history data for prepupae and adults reared on the diet treatments to data recorded for field-collected prepupae and the emergent adults. This comparison allowed us to

determine if any of the diets produced specimens equivalent to those generated in a natural population. Information from this study is important for improving rearing methods, as well as integrating the black soldier fly into current waste management strategies in livestock and poultry facilities.

Materials and Methods

Acquisition of flies. At a 100-layer chicken house at the Coastal Plain Experiment Station (CPES) in Tifton, Georgia, we collected 10 to 20 ovipositing soldier flies. These were assumed to be attempting to oviposit due to their recurrent probing of the manure surface with their ovipositors. They were placed in a 454 ml glass jar with a 2 x 3 cm roll of corrugated cardboard as an oviposition substrate. After 24 hr, the cardboard containing soldier fly eggs was placed in another 454 ml Sweetheart® plastic container (Sweetheart Cup Company, Chicago, Illinois) covered with a paper towel, and stored in a rearing room (27 ^{o}C, 60 to 70% RH, and a photoperiod of 14:10 L:D hr) until larvae emerged 4 d later. Once larvae were observed, 50 g of a 15% protein laying-hen ration feed (Country Acres Feed Company, Inc. Brentwood, MO) at 70% moisture was placed in the container. The larvae were allowed to feed on the medium for 4 d prior to being used in the experiment.

Wild Fly Population. Black soldier fly prepupae were collected from swine and poultry units located in Tifton, Georgia, and held in six 1 L clear Sweetheart® plastic cups (Sweetheart Cup Corporation, Chicago, IL), at a density of approximately 500 prepupae per cup, in the rearing room mentioned above. Prepupal and adult characteristics compared across diet treatments were compared to data recorded for the wild population (see below).

Experimental design. The study examined 3 diets (Table 2-1): (1) CSMA, an artificial rearing media for house fly larvae (Chemical Specialties Manufacturers' Association, Ralston Purina, St. Louis, MO); (2) Gainesville larval diet (Gainesville diet), developed for rearing house flies by Hogsette (1985) and the diet currently suggested for mass rearing the black soldier fly (D.C.S. unpublished data); and (3) the 15% protein laying hen ration feed, which had been used in preliminary trials for rearing *H. illucens*.

The experiment was replicated three times from April through October 1999. For each diet during an experiment, 300 soldier fly larvae were placed in each of three 1-L clear plastic cups. Cups were each covered with a paper towel and held in the rearing room described above. Trial studies using the chicken feed rate of 10 g of feed mixed with 17 ml water per day resulted in the greatest final larval weight. Therefore, this rate was selected as the feeding rate to be used for all three diets. Chicken feed and Gainesville diets mixed with water had initial moisture levels of 70%, while the CSMA diet moisture was 64%. Feeding was terminated in a treatment when a cumulative 40% of the larvae in the 3 cups reached the prepupal stage. However, daily observations continued until all larvae had entered the prepupal stage or died. Prepupae were identified by a change in integument color from larval white to black (May 1961).

Growth of immature stages. Daily larval weight in each treatment was determined by randomly selecting 10 larvae from each cup per treatment and weighing them on a Mettler AT261 DeltaRange® balance. Weighed larvae were returned to their respective cups. Final larval weight was defined as the average larval weight of the ten larval weights recorded on the day when 40% of all individuals in a treatment reached the prepupal stage.

Cups were examined daily for occurrence of prepupae. Prepupae were transferred to another 1 L cup (containing 100 g white sand as a pupation site) designated to hold prepupae from that particular replicate of a diet treatment. Additionally, 3 prepupae per replicate per treatment were weighed daily and placed in petri plates and stored in a freezer at -15 ^{o}C for later determination of calorie content. Cups containing prepupae were covered with 50 x 50 cm of mosquito netting held in place with a rubber band, and were placed in the rearing chamber, and monitored daily for adult emergence. Percent of larvae to reach the adult stage was recorded for each diet treatment but not for the wild population because it was collected when the flies were in the prepupal stage. Days from egg collection to adult emergence were recorded for each reared fly.

Diets consumption and final percent moisture. After all larvae were removed from a container, the remaining residue (consisting of diet residue, excreta, molted skins) was weighed using a Mettler PC4400 DeltaRange® balance scale prior to and after being placed for 2 wk in a Precision Thelco Model 4® gravity convection oven set at 45 ^{o}C. Original and dry weights were compared to determine final percent moisture of the residue from each treatment. Dry residue weight was compared to total dry weight of the diet provided during the experiment to determine percent residue remaining.

Prepupal calorie content. Mean caloric content of prepupae per treatment was determined using bomb calorimetry (combustion) techniques cited in Dutcher (1983). Frozen prepupae were held for one to two weeks, depending on availability of resources needed for the experiment, in a convection oven set at 55 ^{o}C. Individual dried prepupae were then weighed on a Cahn RTL 7000 Electro-balance®, ground to approximately 1 mm pieces, mixed with calorimeter grade benzoic acid (1:10 specimen weight: benzoic

acid weight) and compressed into a pellet. We were unable to make pellets with prepupae collected from the wild. These prepupae, unlike those reared on the diet treatments, appeared to have a greater amount of fats present and attempts to crush a specimen after being dried in the oven resulted in the observable loss of materials from that specimen. Therefore wild prepupae were analyzed without being mixed with benzoic acid. To ensure that this difference in techniques did not influence results generated, calorie content was determined for prepupae from each diet treatment prepared with and without benzoic acid. A Parr® oxygen bomb calorimeter (Model No. 1341) was used to determine caloric content of pellets. Caloric content per mg ash-free dry weight (AFDW) per prepupa per diet treatment and for the wild population was determined.

Selected life-history traits of the adult black soldier fly. Weight, sex, and time from oviposition to adult emergence were recorded for emergent adults. Individual adults were placed in 35-ml Solo® cups (Solo Corporation, Urbana, IL) capped with a breathable lid. Half of the adult flies were provided 0.125 ml water daily via a 1.25 cm needle inserted through the lid. The remainder of flies were held in similar conditions, but without water. Longevity was recorded for both groups.

Egg development and mass size. Initially, we hypothesized that larval media influences the number of eggs produced by adult black soldier flies and that mating was not required to produce eggs. Therefore, in order to determine the number of eggs per individual, a sample of non-mated females from each diet treatment and from the wild population was dissected in saline solution in a petri plate 1, 2, 3, and 4 d after emergence. Ovaries were removed and the number of developed eggs per individual recorded. Specimens were dissected and examined under a Leica® 2000 Zoom dissecting microscope. A fully

developed egg was defined as elongate and creamy to white in coloration, while an oocyte was defined as spherical and transparent. Oviposition by females in 35 ml cups was also recorded to determine if the number of eggs in dissected specimens was similar to the number deposited by females reared on the same diet.

Dissected unmated specimens did not contain developed eggs. Therefore, we suspected that mating is a requirement for egg production. To test this hypothesis, approximately 500 freshly emerged (< 24 hr in age) black soldier flies were collected from a colony (larvae fed Gainesville house fly larval diet) and released at 3 p.m. in a 2 x 2 x 3 m cage in a greenhouse maintained at approximately 30 ^{o}C. Observations and collection of mating pairs were made hourly beginning at 4 p.m on the day the adults were released and continued daily from 8 to 5 p.m. A total of 55 mating black soldier fly pairs were collected by the conclusion of the second day after releasing the adults in the cage. Mated females were weighed within 1 hr of mating, placed in 35-ml cups, and stored in the rearing room previously described and provided water as previously described. We determined in a previous experiment that oviposition generally occurred 2 d after mating (Tomberlin, unpublished data). Therefore, 2 d after mating the ovaries of 45 of the mated females were dissected to determine percent with developed eggs. However, developed eggs per individual were not recorded.

The remaining 10 females were allowed to oviposit in corrugated cardboard taped to the opening of the 35 ml cup. Eggs, when first observed in the cardboard, were removed and number determined based on mass weight divided by individual egg weight as estimated by Booth and Sheppard (1984). Weighed egg masses were then place in 35 ml plastic cups, covered with a breathable lid and placed in the rearing room to hatch.

Estimated number of eggs deposited in the cardboard was compared to number of eggs recorded for dissected mated females.

Egg development studies were problematic because of misconceptions concerning the definition of a black soldier fly egg mass, or eggs oviposited by one individual. Booth and Sheppard (1984) stated that the average black soldier fly egg mass was 998 $\pm$ 78 (mean + SE). They collected eggs using the openings in corrugated cardboard (flutes) as the oviposition site. Prior to Booth and Sheppard (1984), researchers reported black soldier fly egg masses with 119 to 502 eggs (Gonzales et al. 1963) and 205 to 802 (Stephens 1975) in dried hen manure and on bananas, respectively.

Booth and Sheppard (1984) indicated eggs per mass can be estimated by allowing oviposition to occur in cardboard flutes and that the eggs present per flute represent a single mass laid by one individual. Therefore, to determine number of eggs oviposited by adults reared on each of the 3 diets and from the wild population, we released approximately 500, < 24 hrs in age, adults from each treatment into 2 x 2 x 3 m cages placed in the greenhouse previously described. Each treatment had a separate cage. In order to collect egg masses, a 5-L bucket (Plastic Packaging Corporation, West Springfield, MA) containing approximately 1 kg Gainesville diet saturated with water was placed on a cinder block (40 cm height) in each cage to attract individuals ovipositing. Methods for collecting eggs were adapted from Booth and Sheppard (1984). Three layers of corrugated cardboard (each flute opening measured 1 x 4 mm) were glued together, cut into 5.0 x 2.5 x 1.0 cm blocks, and taped inside the 5 L bucket approximately 3 cm above the media. These cardboard blocks were collected, clutches recorded, and replaced daily. An egg mass was defined as a flute containing > 100 eggs.

Egg masses collected each day from each treatment were weighed and stored in vials containing 80% ethanol until the egg number could be recorded.

Data recorded from the experiment described in the previous paragraph indicated that using eggs per flute did not accurately estimate number of eggs oviposited per individual (Table 2-6), but that flute size limits the number of eggs deposited in it. Therefore, we conducted an experiment that examined the relationship between flute dimensions and eggs deposited per flute for black soldier flies reared in the colony at the CPES and held in a 2 x 2 x 3 m cage placed in the greenhouse. We examined three sizes of cardboard flutes; one with a smaller opening (1 x 3 mm); one with the same opening (1 x 4 mm); and the third with a larger opening (2 x 5 mm) than that used in the diet experiments. Cardboard flutes were collected and replaced once daily. Eggs from each flute containing eggs were stored in a vial containing 80% ethanol. Egg number per flute was determined using previously described methods.

Statistical Analysis: Procedure GLM (SAS Institute 1992) was used to analyze the recorded data. Least Significant Difference test (SAS Institute 1992) was used following a significant F test ($P < 0.05$) to separate means between diet treatments and the wild population for: larval and pupal development periods; percent survival to prepupal and adult stages; percents female and male to emerge, final larval, pupal, and pupal skin weights; adult male and female weights; final diet residue moisture; percent residue of dry matter per diet fed to the larvae; and male and female longevity with and without water. Percentage data were arcsine transformed in order to normalize data.

Mean egg number per flute and total weight of eggs per flute were analyzed using the procedures previously described. Regression and Pearson's correlation analysis (SAS

Institute 1992) were used to determine the relationship between egg number and mass weight per flute for each treatment, as well as adult weight and longevity.

Results

Immature Life-History Traits. Final larval weight across treatments ranged from 0.153 to 0.171 g (Table 2-2) and did not differ significantly ($F = 1.71$; $df = 4, 4$; $P > 0.3074$). Survivorship of larvae to the prepupal stage was not significantly different across treatments ($F = 1.07$; $df = 2, 23$; $P > 0.3592$) and ranged from 95.99 to 97.76% (Table 2-3). Time from egg to prepupal stage per treatment ranged from 22.54 to 24.14 d (Table 2-2) and did not differ significantly ($F = 0.74$; $df = 4, 4$; $P > 0.6121$). Mean prepupal weights differed significantly among treatments ($F = 146.79$; $df = 3, 116$; $P < 0.0001$) and ranged from 0.104 to 0.220 g. Pupal skins accounted for about 10% of their weights (Table 2-4). Prepupae reared on chicken feed had the greatest weight among diet treatments, but still weighed significantly less than prepupae from the wild populations. Additionally, prepupal skin weight differed significantly between diet treatments ($F = 75.47$; $df = 3, 156$; $P < 0.0001$) with skins from specimens reared on chicken feed and from the wild population weighing the most.

Mean calorie content per mg AFDW prepupa was significantly different across treatments ($F = 8.70$; $df = 3, 76$; $P < 0.0001$) and ranged from 3.51 to 5.95 cal per mg AFDW with those specimens collected from the wild having the greatest calorie content (Table 2-4). Wild prepupae weighed more and had a higher concentration of fat yielding approximately 3.5 times more calories, than prepupae reared on the diet treatments - prepupae reared on the diets contained approximately 365 calories, while the wild prepupae contained an average of 1309 calories.

Adult Life-History Traits. Mean adult emergence rate (Table 2-3) differed significantly ($F = 26.84$; $df = 3, 27$; $P > 0.0001$) and ranged from 21.74 to 27.20% for the diet treatments, while 91.3% of the prepupae sampled from the wild yielded adults. Of those to emerge, 55.16 to 60.49% were female (Table 2-3) with no significant difference across diet treatments or the wild population ($F = 1.05$; $df = 2, 22$; $P > 0.3660$). In comparison, a sample ($n = 128$) of adults reared from the wild-collected prepupae was similar with 55% emergent adults being female. Male black soldier flies reared on diets were significantly smaller than females ($F = 493.59$; $df = 7, 1854$; $P < 0.0001$) reared on the same diet. This size differential also occurred in the wild population (Table 2-4). However, when comparing the same sex across treatments, wild males and females were significantly larger than their diet-reared counterparts. Although not significantly different ($F = 9.92$; $df = 7, 10$; $P > 0.2875$), adult males generally emerged earlier than females (Table 2-2).

An interaction effect was determined for larval diet and the provision of water on adult longevity ($F = 48.22$; $df = 15, 1764$; $P < 0.0001$) of black soldier flies (Table 2-2). Adult males reared on a diet and provided water as adults lived 9.3 to 9.7 d, while those not provided water lived 5.9 to 7.8 d. Females reared on the treatments and provided water as adults lived 7.9 to 8.5 d, while those not provided water lived 6.1 to 6.4 d. Males and females that emerged from wild-collected prepupae lived significantly longer than those reared on the artificial diets. Wild males and females lived approximately 14.3 d when provided water as adults and 7.8 to 8.2 d when not provided water. Male and female longevity within a treatment when not provided water did not differ.

Additionally, longevity of adults provided water was significantly correlated with individual weight (r^2= 0.40; P < 0.0001).

Egg development and mass size. Twenty virgin females per treatment, and four from the wild population, from 1 to 4 d after emergence were dissected and examined for mature eggs, but none were found (Figure 2-1 and 2-2). For each treatment, including the wild population, less than 1% of the virgin females placed in plastic medicine cups laid eggs. These were monitored for one week, but none hatched. Thirty-two (72%) of the 45 mated females collected from the colony and dissected 2 d after mating (Figure 2-3) had completely formed eggs. Number of eggs per individual ranged from 323 to 621, which accounted for approximately 13 to 26% of female body weight. We were unable to do a correlation analysis between total eggs and body weight of dissected individuals due to our recording data for only five of the dissected individuals containing developed eggs. Six of the 10 mated females collected from the colony and held in 35 ml cups deposited eggs. The number of eggs per individual ranged from 206 to 639, which accounted for 7.9 to 23.4% of the female body weight.

Egg collection system. Mean eggs per flute (F = 0.72; df = 3, 71; P > 0.5456) and weight of eggs per flute (F = 0.83; df = 19, 20; P = 0.4798) were not significantly different among diet treatments, including the wild population (Table 2-6). Individual eggs ranged in weight from 0.023 to 0.025 mg and mean number of eggs per flute ranged from 603 for adults reared on Gainesville diet and the wild population to 689 for adults reared on layer ration feed. Mean weight of eggs per flute ranged from 145.35 mg from adults reared on the Gainesville diet to 159.13 mg for adults reared on the CSMA larval fly diet. Mean weight and number of eggs per flute for adults reared on each diet and the

wild population were significantly ($P < 0.05$) correlated (Table 2-6). The regression analysis for mean egg number versus egg mass weight per flute size was also significant for all treatments including the wild population (Table 2-6).

The inability to determine differences in the size of egg masses per diet treatment and for the wild population was due to the early misconception that one flute held the eggs of one female. Flutes were packed with eggs and the number of eggs deposited per flute is limited by flute size. The number of eggs deposited in the smaller flute (1 x 3 mm) examined averaged 431 eggs per flute, while the larger flute (2 x 5 mm) averaged 1157 eggs per flute. Based on measurements of the depths that eggs were deposited in the different sized flutes, females were able to insert their abdomen and ovipositor approximately one cm into the larger flute to lay eggs, while females were only able to lay eggs at a depth of approximately 0.7 cm in the smallest flute. Mean egg weight between flute treatments was not significantly different ($F = 0.83$; $df = 3, 71$; $P > 0.4798$) from that recorded for eggs deposited by females reared on each of the diet treatments. Six of the 10 mated females held singly in plastic cups deposited between 137 to 639 eggs each, which was 4 to 23% of female body weight. Females dissected 3 d after laying eggs (Figure 2-4) had no additional ovary development.

Diet treatment residue and moisture. Grams of residue remaining for each diet treatment at the conclusion of the experiment did not differ significantly ($F = 1.35$; $df = 2, 24$; $P > 0.2793$) and ranged from 34.70 to 35.29% of total diet dry matter fed to the larvae (Table 2-5) with moisture content of residue being 52% or lower (Table 2-5) and not significantly differing across diet treatments ($F = 1.88$; $df = 2, 24$; $P > 0.1746$). There

was no diet held without larvae since diet reduction due to larval activity was not of interest.

Discussion

All three diets are nutritionally similar, which may explain why final larval weight and caloric content of the reared prepupae were comparable to one another. However, they differed from wild prepupae. This may be due to wild larvae having access to additional nutrients (Slansky and Scriber 1985, Dicke et al. 1989), such as the continuous stream of fresh manure in poultry facilities, not mixed with old resources as in our tests. Recent laboratory studies determined that black soldier fly larvae fed 5-d old hen manure grew at half the rate of larvae fed 18 hr old manure. (D.C.S. unpublished data). Weight differences recorded for prepupae reared on chicken feed and those reared on the CSMA and Gainesville diets may be attributable to differences in calcium content (Table 2-1). Soldier fly larvae sequester ingested calcium and convert it to calcium carbonate, which is secreted through the hypodermis and covers the larval integument (Johannsen 1922).

Development time from egg to prepupae for the black soldier fly in our study was six to eight days shorter than that recorded by May (1961) who reported a mean development of 31 d under similar temperature and humidity conditions. Differences between the two studies may be due to various factors, such as different larval densities (Morrison and King 1977) or food quality (Slansky and Scriber 1985; Dicke et al. 1989). Furman et al. (1959) suggested these factors could delay black soldier fly larval development up to four months. However, May (1961) did not provide sufficient detail to compare these factors.

Differences in percent emergence per diet treatment and those collected from the wild also may be due to larval diet and being held in a sub-optimal habitat. Larvae in our experiment were held and fed in containers that resulted in continuous contact with their waste product. Sheppard (1983) determined that wild soldier fly larvae remain on the surface of the wastes accumulating below layer facilities and feed on fresh manure as it's produced. Manure digested by the larvae then accumulates below the larvae. Additionally, because the soldier fly larvae in our experiment were held in closed containers they fed on media, which was mixed with diet residue and their waste. Therefore, the soldier fly larvae may have been forced to feed to a degree on their own wastes, which we suspect they normally avoid. Larvae of other dipteran species, such as the house fly, need fresh manure daily for optimal growth (Morgan and Eby 1975). This is because anaerobic organisms digest manure as it ages resulting in fewer nutrients for the larvae and thereby suppressing larval growth (Beard and Sand 1973). Development from the egg to the adult stage did not differ between sexes for wild black soldier flies, as well as those reared on diet in the laboratory.

Water, unlike food, is essential for adult black soldier flies to reproduce (D.C.S. unpublished data). This is supported by our data, which demonstrated that individuals provided water lived 1 to 2 d longer than those not provided water. Maintaining a black soldier fly colony without providing water was unsuccessful (D.C.S. unpublished data). This inability to maintain a black soldier fly colony without providing water may be due to the less vigorous dehydrated adults being unable to reproduce effectively and not laying viable eggs.

Wild adult soldier flies provided water lived 4 d longer than adults reared on the diets provided water. This significant difference in longevity may be explained by calorie content of the prepupae. Prepupae reared on the diet treatments contained 0.364 to 0.448 kilocalories, while the wild prepupae contained 0.595 kilocalories per prepupa. The additional calories provided energy that may have resulted in increased longevity of the wild adults.

Eggs masses from diet-reared and the wild adults were smaller than those recorded by Booth and Sheppard (1984), but individual egg size was similar. The reduction in egg number per clutch for our experiment and that recorded by Booth and Sheppard (1984) is most likely due to flute size, which we determined can limit the number of eggs deposited per flute. Therefore, our results really estimate number of eggs per per flute. This also may have been the case for Booth and Sheppard (1984). However, the flute size they used is unknown. Additionally, we suspect it is possible for multiple individuals to deposit eggs in a single flute, which would skew clutch size estimates.

Egg mass weight varied less than 1% when allowing individuals to oviposit in cardboard flutes of equal size. However, egg masses oviposited by mated individuals from the black soldier fly colony and placed in 35 ml cups and the number of eggs per mass from individuals from the colony dissected 2 d after mating varied as much as 75%. We hypothesize dissecting females 2 d after mating provides an accurate estimate of the number of eggs produced by a female. Oviposited eggs may not be as accurate due to variables influencing number of eggs produced, such as time from emergence to mating. Rogers and Marti (1994) determined that the age of female fall armyworms, *Spodoptera*

frugiperda (J. E. Smith), at first mating significantly influences reproductive potential and that extended female virginity resulted in reduced fecundity and fertility.

The black soldier flies in our studies reproduced without feeding and apparently relied on fat reserves acquired during larval development for adult survival and egg production (D.C.S. unpublished data). Therefore, any delay in mating could result in resources being re-allocated from producing eggs to prolonging the adult stage. Similar to our hypothesis for the black soldier fly, Chippindale et al. (1993) determined that food quality directly related to egg production and inversely related to adult longevity. (Diptera: Drosophilidae). Higher quality food resulted in greater egg production, but reduced longevity. Additionally, the number of times a female arthropod mates can influence the number of eggs deposited. As suggested by Foster and Ayers (1995) for the female *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), mating more than once might be attributed to male deficiencies, but may result in greater numbers of eggs being fertilized and deposited.

Most of the previous information on the black soldier was restricted to its use as a biological control agent of house flies and manure management agent in poultry facilities. Our study provides additional information on the life history of black soldier flies reared on three diets, as well as for those collected from the wild. Such information is necessary for developing its potential as a biological control and waste management agent in livestock and poultry production. However, because of the large differences between wild and laboratory-reared specimens, further research is needed to refine and improve larval rearing for mass production of the black soldier fly.

Acknowledgments

We are grateful to G. Herzog for allowing us the use of an environmental chamber and Dr. J. Dutcher for the use of the bomb calorimeter during our experiments. We would also like to thank J. Ruberson, W. Berisford, R. Noblet and J. Butler for reviewing the manuscript.

References Cited

Axtell, R. C. and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annu. Rev. Entomol. 35: 101-126.

Beard, R. L. and D. S. Sand. 1973. Factors affecting degradation of poultry manure by flies. Environ. Entomol. 2: 801-806.

Booth, D. C. and D. C. Sheppard. 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing and site characteristics. Environ. Entomol. 13: 421-423.

Bradley, S. W. and D. C. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. J. Chemical Ecol. 10: 853-859.

Chippindale, A. K. A. M. Leroi, S. B. Kim, and M. R. Rose. 1993. Phenotypic plasticity and selection in *Drosophila* life history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. 6: 171-193.

Dicke, M., M. DeJong, M. P. T. Alers, F. C. T. Stelder, R. Wunderink and J. Post. 1989. Quality control of mass-reared arthropods: Nutritional effects on performance of predatory mites. J. Appl. Ent. 462-475.

Dutcher, J. D. 1983. Ecological energetics and pecan weevil bionomics. J. Ga. Entomol. Soc. 18: 208-213.

Foster, S. P. and R. H. Ayers. 1995. Multiple mating and its effects in the lightbrown Apple moth, *Epiphyas postvittana* (Walker). J. Insect Physiol. 42: 657-667.

Furman, D. P., R. D. Young and E. P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52: 917-921.

Gonzalez, J. V., W. R. Young, and M. R. Genel. 1963. Reduccion de la poblacion de mosca domestica en gallinaza por la mosca soldado en el tropico. Agric. Tec. Mex. 2: 53-57.

Hogsette, J. A. 1985. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. J. Econ. Entomol. 85: 2291-2294.

James, M. T. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bull. Brooklyn Entomol. Soc. 30: 165-170.

Johannsen, O. A. 1922. Stratiomyiid larvae and puparia of the north eastern states. J. N. Y. Entomol. Soc. 30: 141.

Kemppinen, C. S. 1998. An investigation of some factors influencing oviposition and larval development in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Master's Thesis. University of Georgia, Athens, Georgia.

May, B.M. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). N.Z. J. Sci. 4: 55-65.

Morgan, N. O. and H. J. Eby. 1975. Fly protein production from mechanically mixed animal waste. Israel J. Entomol. 10: 73-81.

Morrison, R. K. and E. G. King. 1977. Mass production of natural enemies. In: Biological control by augmentation of natural enemies. Ed. By R.L. Ridgway and S.B. Vinson. Plenum, New York.

Rogers, C. E. and O. G. Marti, Jr. 1994. Effects of age at first mating on the reproductive potential of the fall armyworm (Lepidoptera: Noctuidae). Environ. Entomol. 23: 322-325.

SAS Institute. 1992. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, NC.

Sheppard, D. C. 1983. House fly and lesser house fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1242.

Sheppard, D. C., G. L. Newton and S. A. Thompson. 1994. A value added manure management system using the black soldier fly. Bioresource Technology 50: 275-279.

Slansky, F. Jr. and J. M. Scriber. 1985. Food consumption and utilization. In: Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4: Regulation: digestion, nutrition, excretion. Ed. By G.A. Kerkut and L.I. Gilbert. Pergamon Press, Oxford.

Stephens, C. S. 1975. *Hermetia illucens* (Diptera: Stratiomyidae) as a banana pest in Panama. Trop. Agric. (Trinidad) 52: 173-178.

Tingle, F. C., E. R. Mitchell and W. W. Copeland. 1975. The soldier fly, *Hermetia illucens* in poultry houses in north central Florida. J. Ga. Entomol. Soc. 10: 179-183.

Table 2-1. Composition of chicken layer ration, CSMA diet, and Gainesville larval diet used to rear black soldier flies

Constituent	Chicken layer ration	CSMA	Gainesville larval media
Alfalfa meal	*	27.0	30.0
Wheat bran	*	33.0	50.0
Corn meal	*	___	20.0
Brewers' dried grain	___	40.0	___
Factors			
Protein	15.0	19.0	15.3
Fat	3.0	3.0	3.8
Fiber	5.0	20.0	12.6
Ash	13.7	8.0	6.3
Calcium	5.1	4.0	4.9

* % comparable to that placed in other treatments (information was proprietary)

Table 2-2. A comparison of black soldier fly developmental time (d) to the pupal and adult stages, and longevity for males and females provided and not provided water (mean ± SE), for larvae reared on three different diets at 27 °C, 60 to 70% RH, and a photoperiod of 14:10 (L:D) and for adults reared from field-collected prepupae (n = sample size)[1,2]

Diet treatment	Longevity of adults provided water[3]		Longevity of adults not provided water[3]		Egg to prepupal	Egg to adult	
	Male	Female	Male	Female		Male	Female
Gainesville diet	A9.3 ± 0.4A,a n = 106	A7.9 ± 0.2A,a n = 161	B6.0 ± 0.2A,a n = 159	B6.1 ± 0.1A,a n = 160	22.5 ± 0.7A n=3	43.0 ± 2.9A,a n = 3	43.4 ± 2.1A,a n = 3
CSMA	A9.7 ± 0.4A,a n = 103	A8.5 ± 0.2A,a n = 163	B5.9 ± 0.2A,a n = 145	B6.2 ± 0.2A,a n = 138	23.4 ± 0.3A n = 3	43.0 ± 2.5A,a n = 3	43.0 ± 1.3A,a n = 3
Layer hen ration	A9.3 ± 0.4A,a n = 96	A8.5 ± 0.3A,a n = 151	B7.1 ± 0.2B,a n = 140	B6.4 ± 0.2A,a n = 138	24.1 ± 0.9A n = 160	40.4 ± 2.4A,a n = 3	41.7 ± 2.1A,a n = 3
Wild population	A14.3 ± 1.2B,a n = 25	A14.2 ± 0.9B,a n = 42	B7.8 ± 0.4B,a n = 31	B8.2 ± 0.4B,a n = 22	NA[4]	NA	NA

[1]Means within a column followed by different capital letters differ significantly; [2]Means for both sexes within a treatment with different lower case letters differ significantly ($P < 0.05$; LSD); [3]Longevity means for a sex provided water and not provided water preceded by different capital letters differ significantly; [4]Not available.

Table 2-3. Percent survival of black soldier fly larvae to the prepupal and adult stages, as well as sex ratio (mean ± SE) for larvae reared on three at 27 °C, 60-70% RH, and a photoperiod of 14:10 (L:D), and for prepupae collected from a wild population

Diet	n[1]	Larval survivorship to prepupae	Adult emergence from prepupae	Sex ratio (% female)
Gainesville diet	8	97.8 ± 0.6A[2]	27.2 ± 6.7A	60.5 ± 1.2A
CSMA	9	96.0 ± 1.4A	23.9 ± 4.0A	55.2 ± 2.0A
Layer hen ration	9	96.1 ± 0.4A	21.7 ± 1.8A	58.0 ± 3.4A
Wild population	6	NA[3]	91.3 ± 4.8B	56.0 + 0.0A

[1]Sample size; [2]Means within a column followed by different capital letters differ significantly ($P < 0.05$; LSD); [3]Not available

Table 2-4. Comparison of larval, prepupal, pupal skin, and adult weights (g), and prepupal caloric content per g (mean ± SE) for black soldier flies reared on three different diets (n = sample size) at 27 °C, 60 to 70% RH, and a photoperiod of 14:10 (L:D) and from field-collected prepupae[1]

Diet	Final larval weight	Prepupal weight	Caloric content per mg	Pupal skin weight	Adult weight[2]	
					Male	Female
Gainesville diet	0.157 ± 0.077A n = 3	0.104 ± 0.027A n = 30	3.51 ± 0.28A n = 23	0.011 ± 0.003A n = 40	0.046 ± 0.005A,a n = 272	0.056 ± 0.005A,b n = 339
CSMA	0.153 ± 0.063A n = 3	0.107 ± 0.041A n = 30	4.21 ± 0.39AB n = 22	0.011 ± 0.002A n = 45	0.044 ± 0.005A,a n = 254	0.054 ± 0.005A,b n = 320
Layer hen ration	0.171 ± 0.043A n = 3	0.111 ± 0.034A n=30	4.48 ± 0.27B n = 20	0.016 ± 0.006B n = 45	0.053 ± 0.007B,a n = 234	0.064 ± 0.006B,b n = 316
Wild population	NA[4]	0.220 ± 0.040B n = 30	5.95 ± 0.32C n = 15	0.025 ± 0.008C n = 30	0.085 ± 0.016C,a n = 57	0.111 ± 0.022C,b n = 70

[1] Means within a column followed by different capital letters are significantly different; [2]means for both sexes with different lower case letters within a treatment differ significantly ($P < 0.05$; LSD);

[3]Not available.

Table 2-5. Comparison of percent final moisture and diet residue for three diets (mean ± SE) used to rear black soldier fly larvae in a rearing room set at 27 °C, 60-70% RH, and a photoperiod 14:10 (L:D)[1]

Diet	n	Final % moisture	residue remaining (g)
Gainesville diet	8	52.8 ± 3.5A[2]	34.7 ± 0.7A
CSMA	9	50.4 ± 3.3A	36.3 ± 0.7A
Layer hen ration	9	36.1 ± 7.9A	35.2 ± 0.7A

[1]Means within a column followed by different capital letters differ significantly ($P < 0.05$; LSD)

Table 2-6. Comparison of clutch weight (g), and egg weight (mg) (mean ± SE) for black soldier flies reared on three different diets at 27 °C, 60 to 70% RH, and a photoperiod of 14:10 (L:D), and for field-collected prepupae as well as the correlation and regression analyses for clutch weight (mg) and eggs per clutch per treatment

Treatment	Correlation[2]	Regression models[2] for eggs per clutch	Clutch weight	egg weight
Gainesville Diet	$r^2 = 0.88$, df = 20	= 173.87 + 29372 (clutch weight)	0.0145 ± 0.00012A[3]	0.24 ± 0.012A
n = 20		F = 62.8; df = 1, 18; P < 0.0001		
CSMA	$r^2 = 0.90$, df = 15	= 143.15 + 31935 (clutch weight)	0.0159 ± 0.00018A	0.25 ± 0.011A
n = 15		F = 55.5; df = 1, 13; P < 0.0001		
Layer ration feed	$r^2 = 0.93$, df = 20	= 124.64 + 35346 (clutch weight)	0.0158 ± 0.00012A	0.23 ± 0.008A
n = 20		F = 122.3; df = 1, 18; P < 0.0001		
Wild population	$r^2 = 0.98$, df = 20	= 25.12 + 38887 (clutch weight)	0.0153 ± 0.00012A	0.25 ± 0.004A
n = 20		F = 686.8; df = 1, 18; P < 0.0001		

[1]Sample size; [2]Pearson's correlation, significance set at P < 0.05; [3]Means within a column followed by different capital letters differ significantly

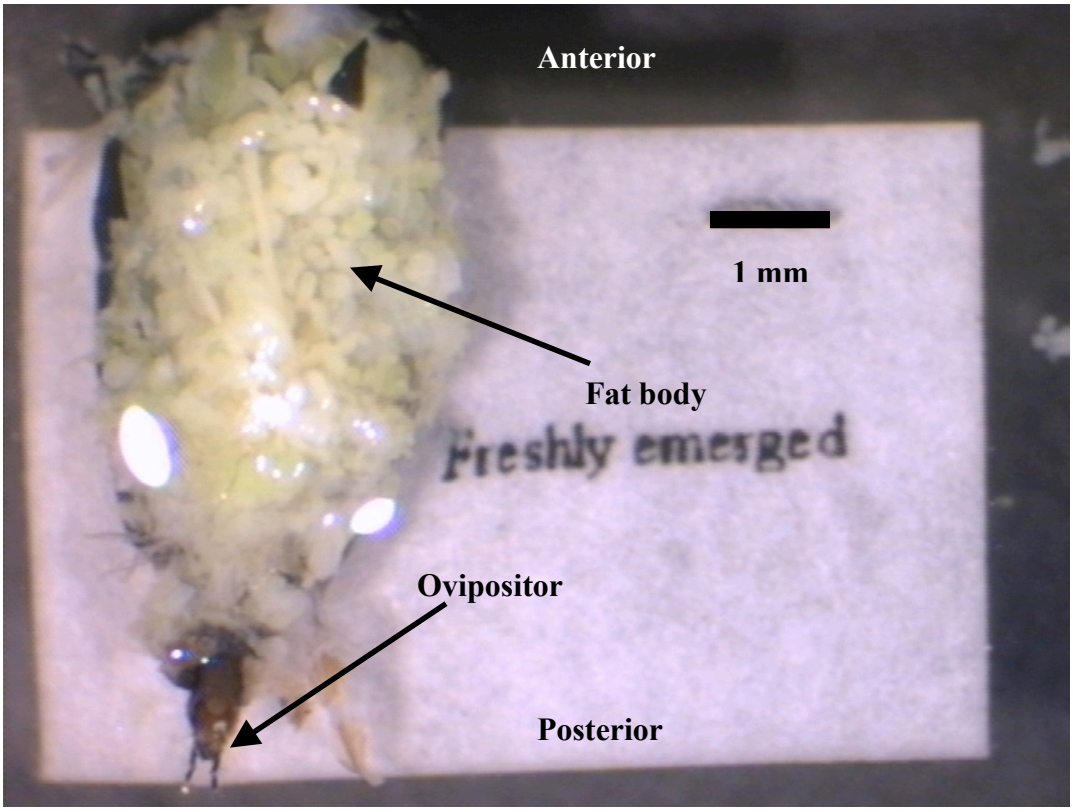

Figure 2-1. Ventral view of dissected abdomen of freshly (< 24 hr) emerged female black soldier fly with fat body present.

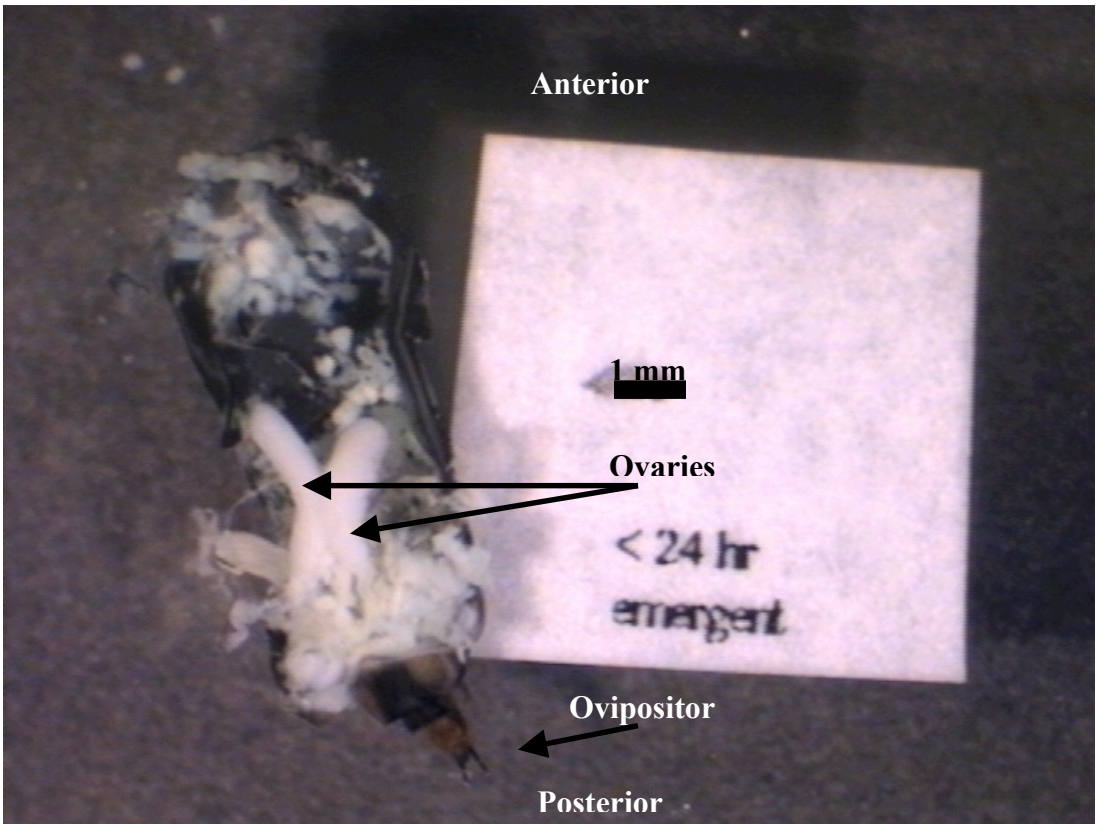

Figure 2-2. Ventral view of dissected abdomen of freshly emerged (< 24 hr) female black soldier fly with fat body removed.

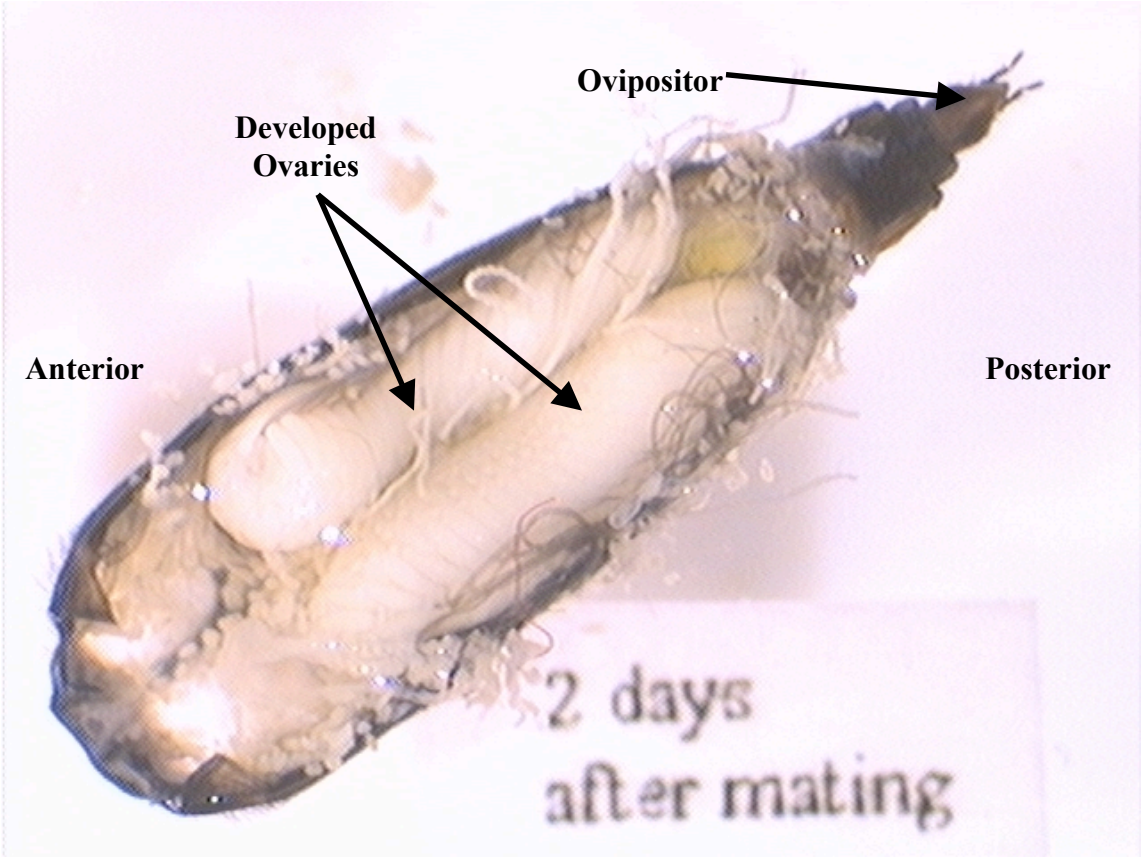

Figure 2-3. Ventral view of dissected abdomen of female black soldier fly 2 d after mating.

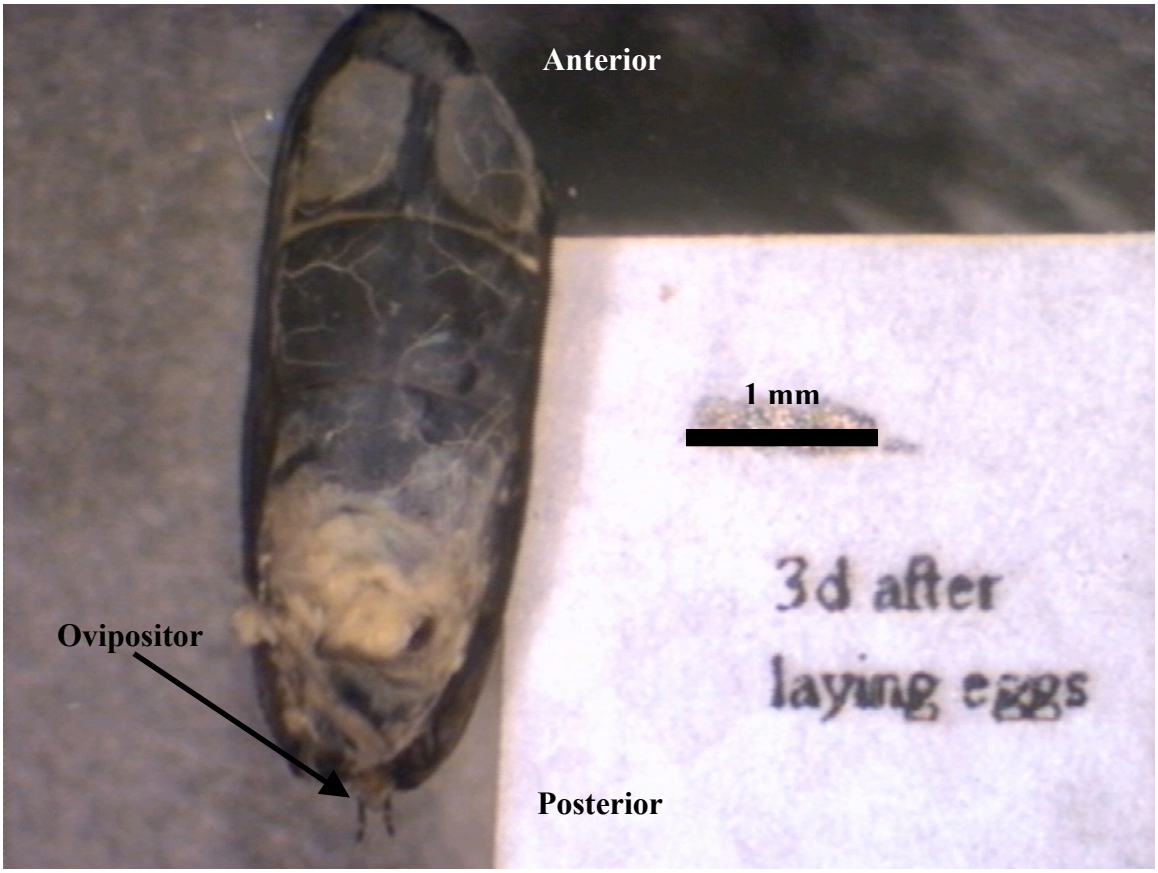

Figure 2-4. Ventral view of dissected abdomen of female black soldier fly 3 d after laying eggs

CHAPTER III

LEKKING BEHAVIOR OF THE BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE)

Jeffery K. Tomberlin and D. Craig Sheppard. Submitted to the Florida Entomologist

The black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae), is a large (13 to 20 mm) and wasp-like fly (May 1961). It has three generations a year (Sheppard et al. 1994) and can be collected from late spring through early fall in the southeastern United States (James 1935). Larvae occur in assorted decomposing materials, such as fruits, carrion, and manure (James 1935).

The black soldier fly has the potential to solve some problems associated with large manure accumulations at confined animal feeding operations (CAFO), such as some pest insects, water pollution and odors. Soldier fly larvae can concentrate excess manure nutrients into valuable feedstuff (ca. $200 per ton) and can be economically transported (unlike manure at ca. $10-20 per ton), which would relieve local nutrient overload (Sheppard & Newton 2000).

The house fly, *Musca domestica* L., may be controlled in manure that is colonized by the black soldier fly (Furman et al. 1959, Tingle et al. 1975, Sheppard 1983, and Axtell and Arends 1990). Additionally, Tingle et al. (1975) documented that black soldier fly larvae reduce waste within poultry facilities. Sheppard (1983) followed up their work and determined that the black soldier fly can reduce manure accumulation by 42-56%. Concentrations of nitrogen, and other nutrients are also significantly lower in this reduced manure, further reducing the potential for pollution, odors, and pathogens.

Soldier fly prepupae can be easily self-harvested by modifying CAFO facilities (Sheppard et al. 1994). Approximately 58 tons of prepupae can be collected in five months from the manure of a single layer facility, housing approximately 100,000 hens (Sheppard et al. 1994). The prepupae can be used as feed (42% protein, 35% fat) for a variety of livestock (Newton et al. 1977, Sheppard et al. 1994). This feedstuff, when

dried, has an estimated value comparable to soybean or meat and bone meal. If used live, as specialty feed, or marketed to exploit its other unique qualities (essential fatty acids and chitin), the value of the product may be higher (Sheppard et al. 1994).

This manure management system depends on a robust soldier fly population for dependable inoculation of the manure with larvae. However, little is presently known about the biology of *H. illucens*.

Tingle et al. (1975) described the black soldier fly mating behavior. Males were attracted to ”calling” females in the same ”resting” area and mating occurred on the ground with the male and female facing opposite directions. However, Copello (1926) noted that mating occurred during flight. We provide a description of the mating, which differs from the description by Tingle et al. (1975), and lekking behaviors of the black soldier fly.

The study site was a poultry farm (three California style open-sided layer houses side-by-side) located in Bacon County, in the coastal plain region of Georgia. Observations were made during the mid-day (1100-1400 hrs) on 21 and 30 July 1998. Various weeds and grasses grew around the poultry facilities with a hardwood forest located approximately 100 m to the north. The forest edge was covered with a mixture of kudzu, *Pueraria lobata* (Wild), and morning glory, *Ipomoea* species. A pond, approximately 15 m in diameter, was located on the eastern side of the forest.

Large numbers of soldier flies were observed at two sites: within the chicken houses (a known larval habitat) and along the edge of the woods, especially a 10 to 15 m sunlit section of kudzu and morning glory facing the poultry facilities. We used an aerial net to sample these areas. We judged that there were several hundred soldier flies present

during our collection periods. Adults collected from the forest edge were 91.9% (n=109) male, while those collected from the poultry facilities were primarily female (91.3%, n=123) apparently seeking oviposition sites. Furman et al. (1959) and Sheppard et al. (1994) suggested that adults live in a wild environment and that those observed in livestock facilities are newly emerged males and females or individuals ovipositing.

Hermetia illucens males present at the forest edge generally rested individually on the surface of leaves. Male movement was observed when conspecifics were present within his vicinity (i.e. flying above the resting male or landing on the same leaf). Arrival of another male would prompt the resting male to close and grapple with the invader prior to landing. This would result in the two vertically spiraling approximately 0.5 to 1.5 m above the resting male's leaf where they would part with one returning to the leaf and the other leaving the vicinity. Females were similarly greeted, however males would grasp passing females during this aerial encounter and descend en copula. No chasing or mating activities were observed at the layer house where ovipositing females predominated.

Similar behaviors have been observed for another stratiomyid species (Alcock 1990). *Hermetia comstocki* Williston males have been observed aggregating at agava trees, *Agave palmeri*, and resting individually on the upper leaf surfaces. Resting males were observed repelling other approaching males. The "victor" of the engagement would return to the leaf while the "loser" would leave the vicinity. This scenario was defined as a territorial or lekking behavior (Alcock 1990). Additionally, these sites of high male density may serve as attractants to females ready for mating (Alcock 1990). Similar

patterns of lekking behavior have been reported for hymenopteran species, and other dipterans (Toft 1989 and O'Neill 1983).

Summary

We describe the lekking behavior of the black soldier fly. If this lekking behavior at specific habitats is needed for *H. illucens* mating to occur, the identification and conservation or creation of these sites near CAFOs would be important. Without these sites, we hypothesize that mating may be reduced or not occur at all, resulting in a reduction in the soldier fly population and associated benefits.

Acknowledgments

We would like to thank J. Ruberson and J. Greene for their helpful comments on this manuscript.

References Cited

Alcock, J. 1990. A large male competitive advantage in a lekking fly*, Hermetia comstocki* Williston (Diptera:Stratiomyidae). Psyche 97: 267-279.

Axtell, R.C. and J.J. Arends. 1990. Ecology and management of arthropods pests of poultry. Annu. Rev. Entomol. 35: 101-126.

Copello, A. 1926. Biologia de *Hermetia illuscens* Latr. Rev. Soc. Ent. Argentina 1: 23 27.

Furman, D.P., R.D. Young, and E.P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52: 917-921.

James, M.T. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae) Bull. Brooklyn Entomol. Soc. 30: 165-170.

May, B.M. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). N.Z. J. Sci. 4: 55-65.

Newton, G.L., C.V. Booram, R.W. Barker, and O.M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44: 395-399.

O'Neill, K.M. 1983. Territoriality, body size, and spacing in males of the bee wolf *Philanthus basilaris* (Hymenoptera: Sphecidae). Behaviour 86: 295-321.

Sheppard, D.C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1442.

Sheppard, D.C. and G.L. Newton. 2000. Valuable by-products of a manure management system using the black soldier fly- A literature review with some current results. Animal, Agricultural and Food Processing Wastes: Proceedings of the 8th International Symposium. James A. Moore ed. American Society of Agricultural Engineers, St. Joseph, Michigan. Pp 35-39.

Sheppard, D.C., G.L. Newton, S.A. Thompson, and S. Savage. 1994. A value added manure management system using the black soldier fly. Bio. Tech. 50: 275-279.

Tingle, F.C., E.R. Mitchell, and W.W. Copeland. 1975. The soldier fly, *Hermetia illucens* in poultry houses in north central Florida. J. Ga. Entomol. Soc. 10: 179-183.

Toft, C.A. 1989. Population structure and mating system of a desert bee fly (*Lordotus pulchrissimus*; Diptera: Bombyliidae). I. Male demography and interactions. Oikos 54: 345-358.

CHAPTER IV

TIME FROM EMERGENCE TO MATING AND OVIPOSITION FOR BLACK SOLDIER FLIES (DIPTERA: STRATIOMYIDAE) IN A COLONY AND INFLUENCE OF CONSPECIFIC LARVAE ON OVIPOSITION BEHAVIOR

Jeffery K. Tomberlin, D. Craig Sheppard, and John A. Joyce. To be submitted to Animal Behavior

Abstract

We examined the time from emergence to mating and oviposition for approximately 750 newly eclosed (< 15 hr), colony-reared black soldier fly placed in a 1.5 x 1.5 x 3 m nylon cage placed in a greenhouse. Sixty-nine percent of mating occurred 2 d after eclosion and 70% of oviposition 4 d after eclosion. Time of day and light intensity were significantly correlated with mating, while time of day, temperature, and humidity were significantly correlated with oviposition.

Oviposition preference was examined for free-flying black soldier flies in a greenhouse colony. Adults were allowed to oviposit in either Gainesville house fly larval diet with and without 5 d old black soldier fly larvae. Based on parametric and sign non-parametric *t*-tests, numbers of eggs were not significantly different between the two treatments.

Introduction

Benefits associated with black soldier fly, *Hermetia illucens* (L.), colonization of manure in poultry facilities are well documented. Larvae in poultry wastes can result in 94-100% suppression of the house fly, *Musca domestica* L., (Sheppard 1983), and a 25% reduction in annual manure accumulation (Sheppard et al. 1994). Prepupae also can be self-harvested and used as a feed for livestock, such as swine (Newton et al. 1977) and fish (Bondari and Sheppard 1981).

Hermetia illucens has a worldwide distribution in the sub-tropics and is active in the southeastern United States from April through October. It will oviposit in a variety of decomposing materials, such as fruit, carrion (James 1935), and manure (Tingle et al. 1975). Eggs are deposited along the edge of manure in poultry facilities

(Sheppard 1983). Eggs hatch after approximately 4 d (Booth and Sheppard 1984) at 27 ^{o}C, 60 to 70% RH and larvae feed for about 2 weeks before becoming prepupae (Tomberlin, unpublished data). Prepupae migrate from the larval habitat (Sheppard et al. 1994) pupate, and adults emerge after about two-weeks depending on environmental conditions (J.K.T., unpub. data). Emerged adults are reported to disperse into the area surrounding and feed on available pollen (Axtell and Arends 1990). Recently, the authors documented a lekking behavior for the black soldier fly (Tomberlin and Sheppard, accepted). Field observations suggest that males aggregate and mate with females that enter the lekking site (Tomberlin and Sheppard, accepted).

The first objective of our study was to determine time from emergence to mating and oviposition for black soldier flies maintained in a colony. The second objective of our study was to determine if female oviposition preferences could be determined for media with and without soldier fly larvae. Such information is essential for colony maintenance and potential mass production of black soldier fly eggs for inoculation of livestock and poultry facilities as a biological control and waste management agent.

Materials and Methods

Time to Mating and Oviposition: Approximately 750 newly emerged (< 15 hr old) adult black soldier flies from the colony were released into an empty 1.5 x 1.5 x 3 m nylon cage located inside the colony greenhouse (6 x 9.4 x 5 m). Flies rested on the cage walls and were watered by an automatic system that sprayed water. Environmental conditions and the number of adults mating and ovipositing were recorded hourly from 0800 to 1800 hrs. Observations were initiated at adult release

and terminated when a complete observation period passed without observing mating and ovipositing. Temperature and humidity were determined with a Hanna HI 9161C® portable microprocessor thermohygrometer and light intensity with a Basic Quantum Meter Model BQM®. All readings were recorded in the center of the cage approximately 30 cm above the ground. Mating was defined as a male and female observed en copula.

In order to determine number of egg masses deposited per hour, a 5 L white bucket containing 1 kg of saturated Gainesville house fly larval diet (Hogsette 1985) was placed in the center of the cage on a 20 cm high cement block for the duration of the experiment. Females oviposited in the flutes of two corrugated cardboard rolls (egg collecting units), measuring 2.54 (diameter) x 4cm (length), taped to the inside of the bucket approximately 3 cm above the moist media. Flute openings measured 2 x 3 mm each. A flute containing > 100 eggs was considered an egg mass. Cardboard rolls were replaced hourly and the number of egg masses recorded.

A stepwise regression (SAS Institute 1992) was used to determine if a significant relationship existed between the number of pairs observed mating and number of egg masses collected with environmental conditions and time of observation. Mating, oviposition, and light intensity data were log10 (n + 1) transformed to stabilize the variances.

Total numbers of females observed mating and egg clutches recorded during the experiment were tabulated and percent occurrence per day determined and regressed with time after female emergence to determine if a significant relationship occurred (SAS Institute 1992). Percent data were arcsine transformed to normalize

data prior to analysis. The experiment was replicated on 3 occasions from February through June, 2000. Light intensity and environmental conditions recorded in the greenhouse did not differ between each experiment.

Oviposition Preference: In the 6 x 9.4 x 5 m greenhouse, two treatments, each consisting of a 5 L white bucket containing 300 g of Gainesville house fly larval media saturated with water, were examined for oviposition preference by black soldier flies. Two egg-collection units, as previously defined, were placed in each bucket approximately 3 cm above the media. According to Booth and Sheppard (1984), black soldier flies prefer to lay eggs in dry sites above the larval medium. Media in one treatment was inoculated with 400 to 500 5 d old black soldier fly larvae approximately 15 minutes prior to initiating the study, while the other was not. Paired treatments were placed on 20 cm high cement blocks at three sites separated by approximately 2 m in the greenhouse (Figure 4-1). A white plastic sheet (12 cm x 6 cm x 1 mm) was placed on top, covered approximately 75% of the opening, in order to shade the media and oviposition sites from direct sunlight. Therefore, 40 to 50 ml of water were mixed daily with the media in each bucket to inhibit soldier fly oviposition in the media. Treatments were replaced every 8 d. Adult flies were released into the greenhouse from the colony to maintain numbers at 500 to approximately 2000 individuals. This variation in fly numbers was due to fluctuating daily fly emergence. The experiment was replicated 8 times sequentially from 26 July to 20 September, 2000.

Egg-collecting units from each treatment, which totaled 6 units per treatment, were replaced and the number of egg masses per treatment recorded daily. Total egg

masses collected from each treatment during the experiment were determined. Initially, a paired *t*-test for independent samples (SAS Institute 1992) was used to determine if significantly more clutches were laid in media inoculated with or without soldier fly larvae. Number of egg masses collected per treatment per day were transformed using a log10 (n + 1). Therefore, a comparison of egg clutches laid per day per replicate was done using a sign non-parametric *t*-test (SAS Institute 1992).

Results and Discussion

Time to Mating and Oviposition: Stepwise regression analysis indicated that hourly environmental conditions significantly influence time of mating (Table 4-1). Light intensity significantly regressed with black soldier fly mating but not with ovipositing females. While investigating a rearing method for the black soldier fly in a rearing room illuminated by several artificial lights maintained at approximately 22 ^{o}C and 60 to 70% RH, we found that mating did not occur and infertile eggs were laid (D. C. S. unpublished data). However, once the soldier flies were exposed to direct sunlight through a 60 x 90 cm window, mated pairs were observed and fertilized eggs were laid.

The eyes of male black soldier flies may be specialized for specific wavelengths in sunlight that were not present in the artificial light. Males of the black fly, *Cnephia dacotensis* (Dyar and Shannon), have a lekking behavior similar to the black soldier fly and is known to be dependent on sunlight to locate mates (McIver and O'Grady 1987). Ommatidia in their eyes have specialized for the detection of the ultraviolet wavelengths, which are abundant in sunlight (Gates 1980).

We determined in a cage test that percent females to mate was significantly correlated to days after emergence (Table 4-1). Sixty-nine percent or more of recorded matings occurred 2 d, and oviposition 4 d after emergence (Table 4-2). Dissected virgin (Figures 2-1 and 2-2) and mated females (Figure 2-3) revealed that oocytes were not present until 2 d after emergence and completely formed eggs 2 d after mating.

Oviposition Site Selection: We determined that oviposition preferences within treatments differed signficantly across replicates ($F = 4.61$; df = 7, 48; $P < 0.0001$), which may be due to variation in environmental conditions recorded in the greenhouse. Humidity ranged from 30 to 85%, while light intensity varied from 3 to 800 μmol $m^{-2}s^{-1}$. Temperature also varied from 24.8 to 38.4 ^{o}C.

We hypothesized that black soldier flies prefer to oviposit on media with black soldier fly larvae. However, for each day, differences in the number of clutches laid in each treatment did not differ significantly ($t = 0.33$; df = 108; $P = 0.7433$). Booth and Sheppard (1984) determined that larval resource type significantly influences oviposition-site selection by gravid females. Additionally, they suggested that the presence of soldier fly larvae could also influence site selection. Our results however did not support their hypothesis.

In conclusion, our results suggest that mating and oviposition behaviors are mediated by environmental cues. However, optimal conditions for these behaviors to occur are not known. Such information would be useful in improving current black soldier fly rearing methods. Such information would also provide insight to cues regulating mating and oviposition behaviors. Additionally, no discernible differences

were recorded for number of egg clutches laid in Gainesville media inoculated and not inoculated with black soldier fly larvae. Therefore, it is not essential to inoculate media with larvae in order to collect soldier fly eggs from a colony or the field.

Acknowledgements

We would like to thank W. Berisford, R. Noblet, J. Butler, and J. Ruberson for their helpful comments on this manuscript

References Cited

Axtell, R. C. and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annu. Rev. Entomol. 35: 101-126.

Bondari, K. and D. C. Sheppard. 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Booth, D. C. and D. C. Sheppard. 1984. Oviposition of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae): Eggs, masses, timing, and site characteristics. Environ. Entomol. 13: 421-423.

Gates, D. M. 1980. Biophysical Ecology. Springer-Verlag, New York and Berlin.

Hogsette, J. A. 1985. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. J. Econ. Entomol. 85: 2291-2294.

James, M. T. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bull. Brooklyn Entomol. Soc. 30: 165-170.

McIver, S. B. and G. E. O'Grady. 1987. Possible role of the central retinular cells in the ommatidia of male black flies (Diptera: Simuliidae). Can. J. Zool. 65: 3186-3188.

Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Sci. 44: 395-400.

SAS Institute. 1992. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, NC.

Sheppard, D. C. 1983. House fly and lesser house fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1242.

Sheppard, D. C., G. L. Newton and S. A. Thompson. 1994. A value added manure management system using the black soldier fly. Bioresource Technology 50: 275-279.

Tingle, F. C., E. R. Mitchell, and W. W. Copeland. 1975. The soldier fly, *Hermetia illucens*, in poultry houses in north central Florida. J. Ga. Entomol. 10: 179-183.

Table 4-1. Equations for black soldier fly mating and oviposition response with temperature, humidity, light intensity, time after emergence for individuals maintained in a greenhouse colony

		Equation of fitted response
Number of	matings	$y = 0.51 - 0.01(h) + 0.31(\log10\ (l + 1))$; $r^2 = 0.49$; $P < 0.0001$
	egg clutches laid	$y = -0.78 + 0.01(h) - 0.01(hum) + 0.03(t)$; $r^2 = 0.58$; $P < 0.0001$
Percent[1] of	females to mate	$y = -579.33 + 1006.40(x) - 530.38(x^2) + 112.01(x^3) - 8.3(x^4)$; $r^2 = 0.99$; $P < 0.0001$
	oviposition	$y = -387.2 + 789.04(x) - 531.89(x^2) + 143.15(x^3) - 13.03(^4)$; $r^2 = 0.99$; $P < 0.0001$

[1]Percent data arcsine transformed prior to analysis; h, hour of day; l, light intensity (μmol $m^{-2}s^{-1}$); hum, humidity; t, temperature; x, day after emergence

Table 4-2. Time (days) from emergence to mating and oviposition ± SE (n = 3) for 750 adult black soldier flies released in a 1.5 x 1.5 x 3 m nylon cage placed in a 6 x 9.4 x 5 m greenhouse in Tifton, Georgia[1]

Days after emergence	% Mating	% Ovipositing
1	7.8 ± 7.5ab	0 ± 0a
2	68.8 ± 6.8c	0 ± 0a
3	16.70 ± 2.1a	12.9 ± 10.3b
4	2.9 ± 1.2b	72.9 ± 11.12c
5	3.7 ± 0.2b	12.9 ± 3.2b
6	0 ± 0b	1.2 ± 1.2a

[1]Means within a column followed by different capital letters differ significantly ($P < 0.05$; LSD)

Table 4-3. Mean number of egg clutches ± SE deposited in Gainesville diet inoculated, and not inoculated with, black soldier fly larvae placed in a 6 x 9.4 x 5 m greenhouse in Tifton, Georgia[1]

Day	n	Egg clutches collected from media inoculated with soldier fly larvae	Eggs collected from media not inoculated with soldier fly larvae
1	8	10.57 ± 4.95a	4.43 ± 2.64a
2	8	29.14 ± 9.13a	22.86 ± 10.06a
3	8	27.86 ± 9.56a	27.28 ± 12.32a
4	8	31.71 ± 8.27a	30.85 ± 9.19a
5	8	25.14 ± 6.65a	41.43 ± 15.69a
6	8	23.43 ± 7.28a	26.29 ± 8.89a
7	7	31.00 ± 9.27a	23.33 ± 6.68a
8	8	15.43 ± 3.24a	28.00 ±5.83a

[1]means across treatments on the same day followed by different lower case letters differ significantly ($P < 0.01$; LSD)

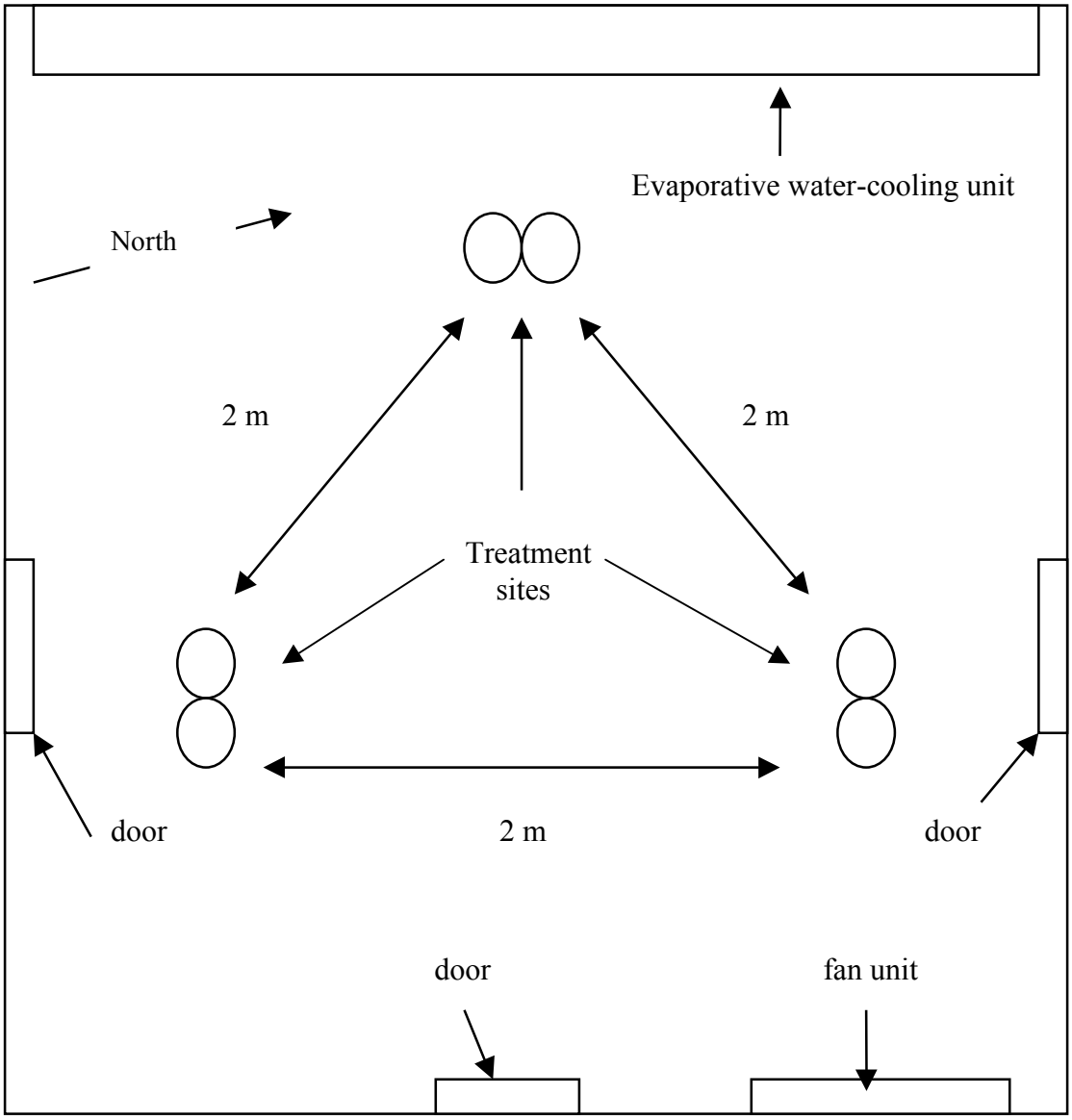

Figure 4-1. Diagram of the 6 x 9.4 x 5 m greenhouse (not to scale) used to house the black soldier fly colony and to examine oviposition site finding behavior. Two treatments were paired at three sites.

CHAPTER V

OLFACTORY AND TACTILE INHIBITION OF HOUSE FLY (DIPTERA: MUSCIDAE) OVIPOSITION BY BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE) LARVAE

Jeffery K. Tomberlin and D. Craig Sheppard. To be submitted to the Journal of Entomological Science

Abstract

Olfactory experiments measuring the response of gravid house flies, *Musca domestica* L., to CSMA house fly larval media inoculated with black soldier fly, *Hermetia illucens* (L.), larvae were conducted.

In a binary choice experiment, olfactory attraction of house flies to CSMA house fly larval diet with and without soldier fly larvae, insect-free cow manure, and spent larval diet prepared 24 hr prior to being examined did not differ significantly. Olfactory attraction levels for treatments prepared 1 hr prior to being examined differed significantly from one another, but not from paired controls. The level of house fly attraction did not significantly differ in a multi-choice test using CSMA diet inoculated (< 1 hr) with 10 densities of black soldier fly larvae.

Because results recorded from the olfactory experiments were inconclusive, we replicated a previous oviposition bioassay that suggested black soldier fly larvae were not attractive, but repelled, ovipositing house flies. Using a previous experimental design with slight modifications, we determined that tactile cues may inhibit house fly oviposition. Our results also support the idea that soldier fly larvae release volatile chemicals (allomones) that inhibit house fly oviposition. However, our results are preliminary and additional olfactory research is needed to decipher the interactions between ovipositing house flies and black soldier fly larvae.

Additionally, olfactometer tests registered any fly approaching the odor source as an attraction, whereas the oviposition bioassay only registered the greater commitment of oviposition as a response. This latter response may be the more appropriate one to evaluate in future studies examining house fly repellency.

Introduction

The black soldier fly, *Hermetia illucens* (L.), is a biological control agent of house flies, *Musca domestica* L., in poultry facilities (Furman et al. 1959; Sheppard 1983). Moon (1980a) hypothesized black soldier fly larvae out-compete house fly larvae for resources. Larval habitats colonized by the black soldier fly eventually become unsuitable for the growth and development of house fly larvae (Moon 1980b).

Bradley and Sheppard (1984) suggest black soldier fly larvae also may release chemicals (allomones) that repel ovipositing house flies. They determined that gravid house flies were able to differentiate between resources in which soldier fly larvae were present or absent, and oviposited significantly more eggs in poultry manure without soldier fly larvae.

Our initial objective was to examine the olfactory response of gravid house flies to CSMA diet containing black soldier fly larvae. For our second objective, we conducted house fly oviposition bioassays similar to those of Bradley and Sheppard (1984), in order to determine the oviposition response of gravid house flies to poultry manure inoculated with various densities of black soldier fly larvae.

Materials and Methods

Olfactometer experiments. We conducted three experiments examining house fly olfactory response to soldier fly larvae in a Medusa® aluminum, multi-port, pie-shaped olfactometer located in the Department of Entomology and Nematology at the University of Florida in Gainesville, Florida (Burkett 1998). For experiment one and two, the olfactometer was portioned into five equally sized wedges for a binary choice test. Each wedge contained one port connected to a treatment cup and another to a control cup.

Treatments for experiment one were prepared 24 hr prior to testing, while those in experiments two and three were prepared one hr prior to the test. Fifteen 10-d-old gravid house flies were placed in each wedge of the olfactometer at the initiation of the experiment and allowed to move freely throughout the wedge. Sixty grams of 3-d-old CSMA larval house fly diet was used as a control paired with each treatment, while the treatments for experiment one were as follows: (1) 10 and (2) 100 soldier fly larvae in 60 g of 3 d old CSMA diet (3) 100 soldier fly larvae without diet (4) 60 g spent media and (5) 60 g insect-free fresh cow manure. Spent media was the diet residue remaining after rearing black soldier fly larvae to the prepupal state in our colony maintained in Tifton, Georgia. For experiment two, the control was the same as that used in experiment one and the treatments were as follows: (1) 5, (2) 10, (3) 30 soldier fly larvae in 60 g 3 d old CSMA diet, (4) 30 soldier fly larvae with no media, and (5) spent media. Soldier fly larvae used in the experiments were 5 to 7 d old and weighed approximately 0.035 g.

During the third experiment, the olfactometer was not partitioned into wedges and all 10 ports were accessible to 50 gravid house flies placed in the olfactometer. Treatments contained 60 g of 3 d old CSMA media with 1 of 10 soldier fly larval densities (0, 5, 10, 25, 40, 60, 70, 80, 90, 100). Controls were the same as those used in previous experiments. The port initially chosen for treatment one (no larvae) was randomly selected. Additional treatments were assigned to remaining ports in ascending order from left to right of the first treatment to reduce selection variability of the house flies.

Treatments and controls for all experiments were sealed in separate 454 ml Sweetwater® plastic cups (Solo Corporation, Sweetheart Cup Company, Chicago,

Illinois). Each cup had two 10 cm sections of Tygon® tubing (1.25 cm diameter) inserted through separate holes near the center of a lid. One section of tubing, attached to a CO_2 tank, mixed CO_2 (0.51 L per min) with odors released by the treatment or control. The CO_2-treatment odor mixture exited through the second section of tubing and entered one of 10 ports spaced at 10 cm intervals along the side of the olfactometer. A central exhaust valve, located on the top-center of the olfactometer, and allowed air to exit the system at a constant rate. Sensors placed over each port opening and connected to a computer recorded house fly contact with ports. Contact with the sensors resulted in the circuit being closed. However, a sensor could not record the number of flies on it at any given time. Visual observations were not made. Experiments were conducted for 4 hr.

Total time that a sensor was contacted was interpreted as house fly attraction to odors being emitted from that respective port. An ANOVA was used to determine if treatments differed significantly. Contact-second means were compared across treatments using Least Significant Difference (LSD) following a significant F test ($P < 0.01$) (SAS Institute, 1992). Data sum for each variable were square root transformed (SAS Institute 1992) prior to analysis. For experiments with significant differences between treatments, a paired *t*-test for independent samples (SAS Institute 1992) was used to determine if the treatments differed from their paired controls. Each experiment was replicated three times.

Oviposition bioassays. We conducted bioassays similar to Bradley and Sheppard (1984). Our experiment had four, instead of three, treatments and three replicates (instead of one) of each treatment were placed with 150 (100 ♀: 50 ♂) 8-d-old adult house flies in a 0.75 m^3 screened caged placed in a rearing room (26 ^oC, 60-70% RH,

14:10 L:D). House flies were from a colony established from adults collected from a poultry facility in Hoboken, Georgia with a robust soldier fly population. Bradley and Sheppard (1984) reported that a wild house fly strain was more sensitive to black soldier flies than was an older, more domesticated strain. Flies were replaced in each replicate.

Four different treatments were provided as oviposition sites. Each had three 454 ml clear plastic cups containing 75 g of insect-free poultry manure at 81% moisture. Treatments one through three were inoculated with 0, 10, or 100 third to fifth instar soldier fly larvae. Manure in treatment four was separated into two layers by a polystyrene disposable sterile petri plate (VWR brand, West Chester, Pennsylvania) with a screened hole (2.54 cm diameter) in the center, which was assumed to allow air to circulate between layers. The bottom layer contained 25 g manure and 100 soldier fly larvae and the top contained 50 g manure. The petri plate acted as a barrier preventing the larvae from disturbing the top layer of manure. If the assumed air flow to the upper manure occurred, then the house flies could only receive olfactory stimuli from the soldier fly larvae.

Treatments were placed in the cage in a randomized block design separated from each other by approximately 8 cm. Black soldier fly larvae were allowed to acclimate to the treatments for 30 minutes. After this 30 minutes, house flies were introduced and allowed to oviposit in the treatments for 24 hr. Treatments were then taken from the cage and soldier fly larvae carefully removed to avoid possible damage to house fly eggs. Treatments were covered with a paper towel, held in place with a rubber band, and placed in the rearing room for 3 d. House fly larvae and pupae in each cup were then recorded. The test was repeated on three occasions. Procedure GLM (SAS Institute 1992) was used

to evaluate and compare the number of immature house flies collected in each treatment. Data were log10 (n + 1) transformed prior to analysis. Least Significant Difference test (SAS Institute 1992) was used following a significant F test ($P < 0.05$) to separate mean number of house fly larvae per treatment.

Results and Discussion

Olfactometer results were inconclusive. Experiments one and two examined treatments paired with controls. Flies (Figure 5-1) in experiment one (treatments prepared 24 hr prior to test) did not differ significantly in their response to any treatment ($F = 0.67$; df = 9, 18; $P = 0.5854$), while flies in experiment two (treatments prepared < 1 hr prior to the experiment) did ($F = 5.06$; df = 9, 18; $P = 0.0012$). However, when treatments from experiment two were compared to their respective controls with a paired *t*-test, no significant differences ($P < 0.01$) were determined (Figure 5-2). Results of the third olfactory experiment (Figure 5-3) were not significant ($F = 1.30$; df = 9, 18; $P = 0.3021$).

We may have selected the wrong larval resource as an attractant for ovipositing house flies in the olfactometer experiments. Gaseous products released by CSMA medium are probably different from those released by manure, which is the primary material colonized by house flies in poultry facilities. According to D'Amato et al. (1980) and Grodowitz et al. (1987), manure produced by cows fed different grain regimens had different physicochemical properties, which they attributed to differences in microbial fauna. Accordingly, Broce and Haas (1999) suggested that physicochemical properties and age of manure determined the level of attraction recorded for ovipositing flies.

The criteria for attraction were different in the olfactometer experiments than in the oviposition bioassays. Movement towards the odor plume would elicit a positive response in the olfactometer experiment. This movement may or may not have been for the purpose of oviposition in the medium producing the odor. In the oviposition bioassay, immatures resulting from oviposition were counted. This gives a much more definitive answer to the question attraction and acceptance of any given treatment as an oviposition medium.

In the oviposition bioassays, house flies laid significantly ($F = 6.49$; $df = 3,56$; $P = 0.0008$) fewer eggs in manure inoculated with soldier fly larvae than in media not containing larvae (Figure 5-4). The mean number of eggs deposited in the treatment with soldier fly larvae isolated in the bottom of the container was greater than that recorded for the treatment containing the same number of soldier fly larvae but able to migrate throughout the manure in which oviposition occurred. Additionally, the treatment with the isolated larvae did receive significantly fewer eggs than the zero larval treatment. Since movement of the manure could not be a factor here, then chemical repellants (allomones) from the black soldier fly larvae may have affected the oviposition of house flies and should not be ruled out.

Number of immature house flies collected from treatments containing different densities of soldier fly larvae did not differ significantly, which differed from results recorded by Bradley and Sheppard (1984). They, unlike us, recorded a significant decrease in the number of house fly eggs and larvae collected in manure inoculated with 10 and 100 soldier fly larvae. Additionally, unlike Bradley and Sheppard (1984), we did not record complete repellency of house fly oviposition by black soldier fly larvae in

poultry manure. However, this difference may be due to difference in experimental design. Unlike Bradley and Sheppard (1984) who placed one replicate of each treatment in the cage, we placed three, which may have diluted odors released from the treatments and prevented the house flies from distinguishing treatments.

Our results suggest that olfactory cues play a significant role in inhibiting house fly oviposition in media inoculated with black soldier fly larvae. However, if inhibition is due to allomone produced by the black soldier fly larvae, studies will be needed to determine their chemical components for possible synthesis and production for use as repellents.

Acknowledgements

We would like to thank J. Ruberson, W. Berisford, J. Butler and R. Noblet for their helpful comments on this manuscript.

References Cited

Bradley, S. W. and D. C. Sheppard. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. J. Chem. Ecol. 10: 853-859.

Broce, A. B. and M. S. Haas. 1999. Relation of cattle manure age to colonization by stable fly and house fly (Diptera: Muscidae). J. Kansas Entomol. Soc. 72: 60-72.

Burkett, D.A. 1998. Light Color Attraction and Dietary Sugar Composition for Several Mosquito (Diptera: Culicidae) Species Found in North Central Florida. Ph.D. Dissertation. Department of Entomology, University of Florida, Gainesville, Florida. 139 pp.

D'Amato, L. A., F. W. Knapp, and D. L. Dahlman. 1980. Survival of the face fly in feces from cattle fed alfalfa hay or grain diets, effect of fermentation and microbial change. Environ. Entomol. 9: 557-560.

Furman, D.P., R.D. Young, and E.P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52: 917-921.

Grodowitz, M. J., A. B. Broce, and L. H. Harbers. 1987. Characteristics of dung that affect face fly, *Musca autumnalis* (Diptera: Muscidae), larva survival and puparial mineralization. Environ. Entomol. 16: 722-730.

Hammack, L. 1991. Oviposition by screwworm flies (Diptera: Calliphoridae) on contact with host fluids. J. Econ. Entomol. 84: 185-190.

Moon, R. D. 1980a. Biological control through interspecific competition. Environ. Entomol. 9: 723-728.

Moon, R. D. 1980b. Effects of larval competition on face fly. Environ. Entomol. 9: 325-330.

SAS Institute. 1992. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, NC.

Sheppard, D. C. 1983. House fly and lesser house fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439-1242.

Sheppard, D.C., G.L. Newton, S.A. Thompson, and S. Savage. 1994. A value added manure management system using the black soldier fly. Bio. Tech. 50: 275-279.

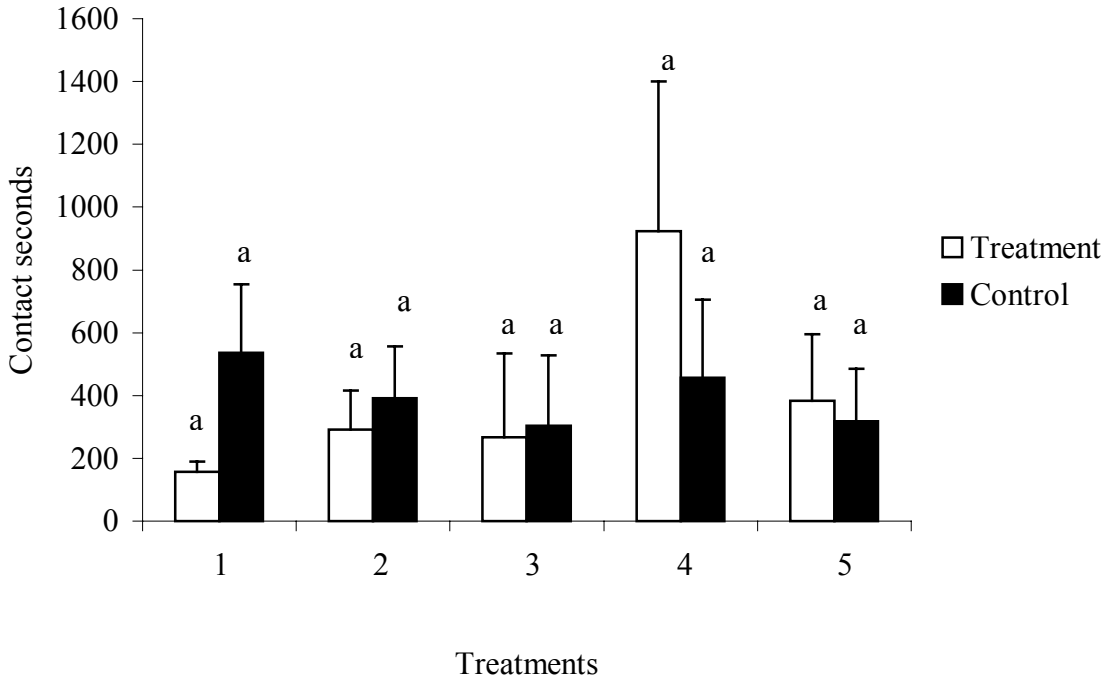

Figure 5-1. House fly mean (n = 3) contact seconds per 4 hr (mean ± SE) exposed to black soldier fly treatments paired with a control in a Medusa® olfactometer. Treatments were as follows: 60 g 3 d old CSMA house fly larval media with (1) 10 or (2) 100 5 d old black soldier fly larvae; (3) 100 black soldier fly larvae with no media; (4) Spent media treatment, which was 60 g of CSMA media that remained 5 d after being fed to soldier fly larvae; (5) 60 g insect-free cow manure. Controls were 60 g of 3 d old CSMA media. Treatments and controls were prepared 24 hr prior to initiating experiment. Means in each pair with different letters are significantly different ($P < 0.01$). Data were square root transformed prior to analysis.

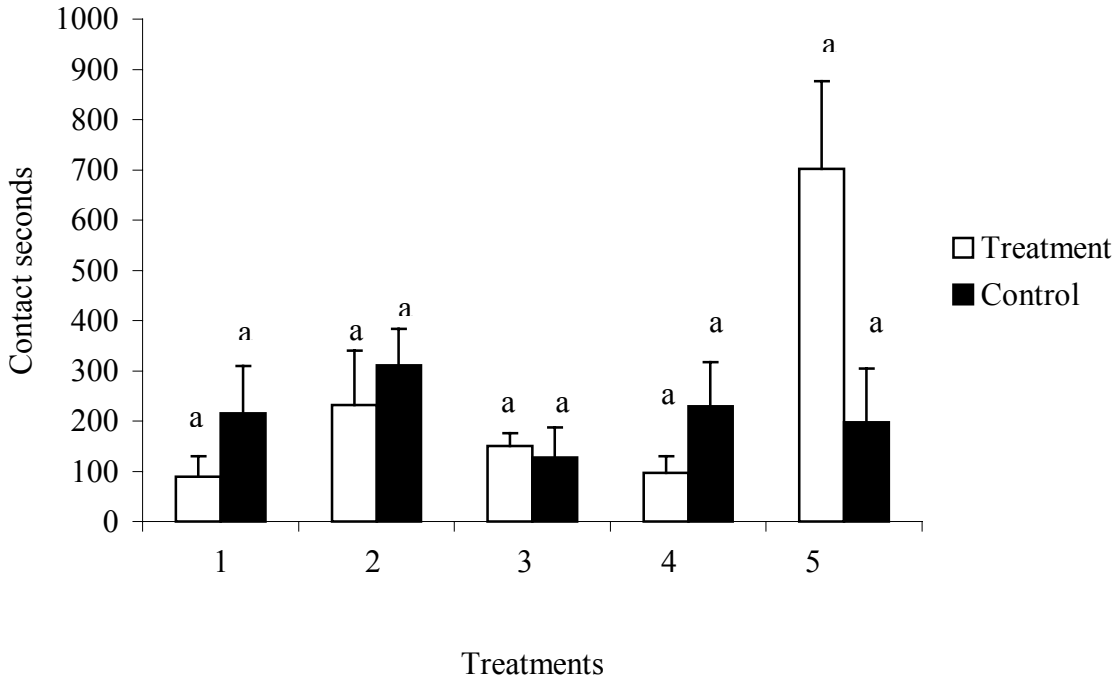

Figure 5-2. House fly mean (n = 3) contact seconds per 4 hr (mean $\pm$ SE) exposed to odors emanating from black soldier fly treatments paired with a control in a Medusa® olfactometer. Treatments were as follows: 60 g 3 d old CSMA house fly larval media with (1) 5 (2) 10 or (3) 30 5 d old black soldier fly larvae; (4) 30 larvae with no CSMA media; (5) Spent media treatment, which was 60 g of CSMA media that remained 5 d after being fed to soldier fly larvae. Treatments were paired with a control, which consisted of 60 g 3 d old CSMA larval house fly media. Treatments and controls were prepared 1 hr prior to initiating experiment. Means in each pair with different letters differ significantly ($P < 0.01$). Data were square root transformed prior to analysis.

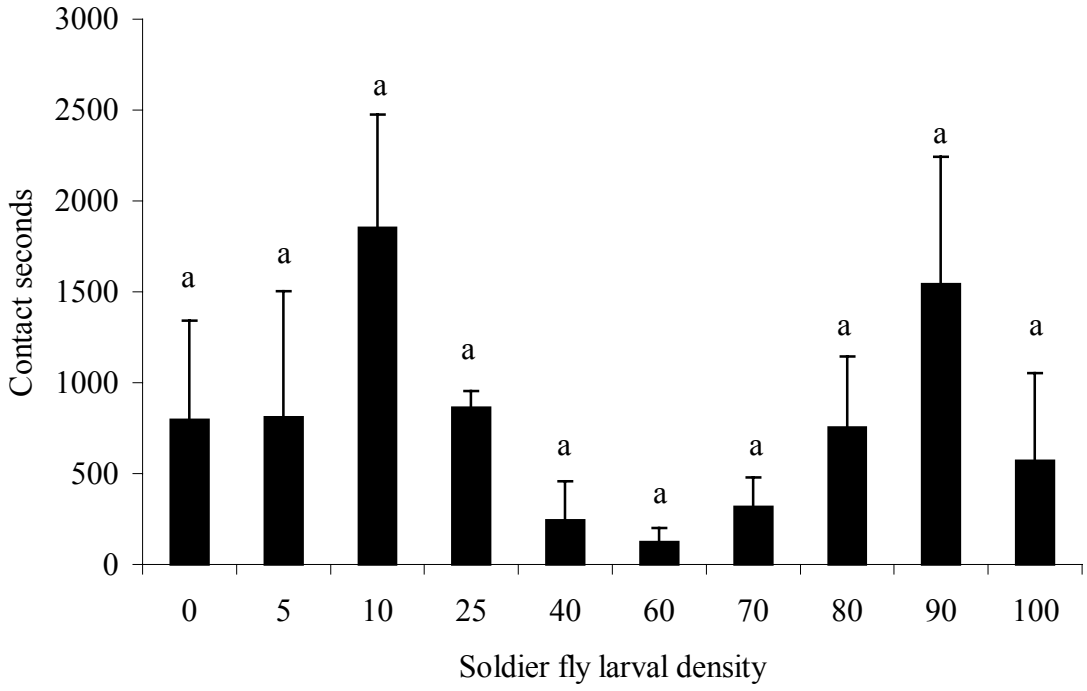

Figure 5-3. Mean (n = 3) house fly contact seconds per 4 hr (mean $\pm$ SE) with odors emanating from 60 g 3 d old CSMA house fly larval media inoculated with 5 d old soldier fly larvae, densities ranging from 0 to 100 larvae, in a Medusa® olfactometer. Treatments and controls were prepared 1 hr prior to the experiment. Procedure ANOVA was used to determine significant differences between mean contact-seconds per treatment ($P < 0.01$, LSD). Data were square root transformed prior to analysis. Means with different letters differ significantly.

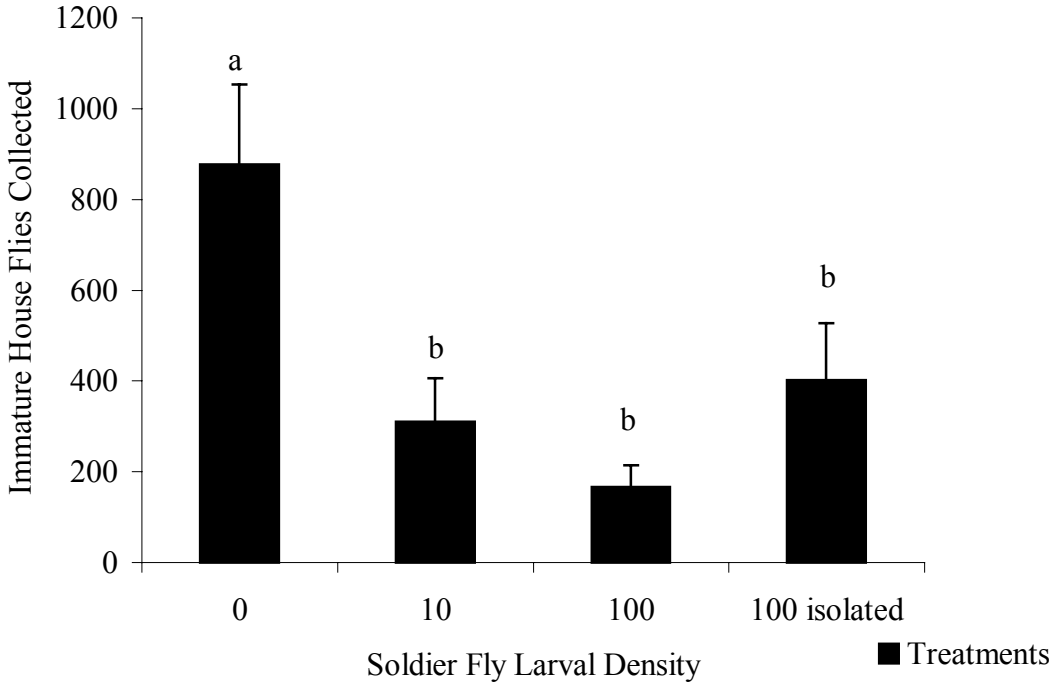

Figure 5-4. Mean (n = 3) number of house fly larvae and pupae collected from 75 g insect-free chicken manure inoculated with various densities of black soldier fly larvae. The fourth treatment (100 isolated) had 50 g manure placed on top of 100 soldier fly larvae with 25 g manure. The layers were separated by a petri plate, which had a 2.54 cm diameter screened hole in its center to allow air to circulate between layers. Means were log10 (n + 1) transformed prior to analysis. Means with different letters are significantly different ($P < 0.01$; LSD).

CHAPTER VI

BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE) LARVAL AND ADULT SUSCEPTIBILITY TO FOUR INSECTICIDES

Jeffery K. Tomberlin, D. Craig Sheppard and John A. Joyce. To be submitted to the Journal of Entomological Science.

Abstract

Dosage-mortality regressions were determined for black soldier fly larvae, *Hermetia illucens* (L.), fed media treated with cyromazine or pyriproxifen. Cyromazine LC_{50}s for larvae failing to reach the adult stage ranged from 0.13 to 0.19 ppm with dosage-mortality regression slopes between 3.94 and 7.69. Cyromazine exposed larvae surviving to the prepupal stage had reduced weights.

Pyriproxifen dosage-mortality regressions were not generated for percent larvae failing to reach the prepupal stage since < 32% mortality was recorded at the highest rate of 1857 ppm. Rates from 0.03 to 232 ppm resulted in 12 to 29% greater prepupal weights than the control, while those rates > 232 ppm resulted in prepupal weights similar to the control.

Forty-seven percent of the control larvae in the pyriproxifen experiment did not emerge as adults. Fifty-seven percent of larvae fed media treated with 0.0625 ppm pyriproxifen failed to reach the adult stage. This was the lowest rate administered. Rates > 58 ppm resulted in $\geq$ 16.7% of surviving larvae not moulting into prepupae after 38d. All larvae exposed to rates < 58 ppm reached the prepupal stage after 38 d. LC_{50}s for larvae failing to become adults ranged from 0.10 to 0.12 ppm with dosage mortality-regression slopes between 1.67 and 2.32.

Dosage-mortality regressions were determined for adult black soldier flies and house flies from wild strains colonized from a poultry facility in southeast Georgia and for a Cornell-susceptible house fly colony maintained in our laboratory. Adult flies were exposed to various rates of two pyrethroids; λ-cyhalothrin and permethrin. Results

generated for the wild soldier flies and house flies were compared to those determined for Cornell-susceptible house flies exposed to the same pesticides.

Our results demonstrate that the wild house fly, unlike the black soldier fly, population was highly resistant to each of these pyrethroids. Regression slopes for black soldier flies exposed to λ-cyhalothrin were twice as steep as those determined for the "wild" house fly strain. Accordingly, LC_{50}s for the black soldier fly and the Cornell-susceptible strain were 10 to 30 fold lower than those determined for the wild house fly strain. The differential sensitivity between the wild black soldier flies and house flies may be affected by behavioral differences. House flies can remain in facilities as adults with the possibility of every emergent adult being exposed to pesticides. Black soldier fly adults are typically present briefly only during adult emergence and oviposition thereby limiting their exposure to the pesticides.

Introduction

Methods for suppressing house flies, *Musca domestica* L., in poultry facilities include biological, cultural, and chemical methods (Axtell and Arends 1990). The black soldier fly, *Hermetia illucens* (L.), can suppress house fly populations up to 94 to 100% and reduce manure accumulation by 42 to 56% (Sheppard 1983). Additionally, 58 tons of 44% dry matter black soldier fly prepupae can be self-harvested in 5 months from a 100,000 hen caged layer house with no external energy inputs and can be used for livestock feed or other products (Sheppard et al. 1994). The black soldier fly is distributed throughout the tropical and subtropical regions of the world, including the southeastern United States (James 1935 and 1947), where it is active from April to November (Sheppard et al. 1994).

Insecticides used against pest populations can disrupt the life cycle of biological control agents, resulting in an increase of pest numbers (Van Driesche and Bellows 1996). For example, Axtell and Edwards (1970) determined that using insecticides in poultry facilities to suppress black soldier flies resulted in a resurgence of house flies.

This study was conducted to determine the response of black soldier fly larvae and adults to some insecticides used for house fly control in poultry facilities. This information could allow the effective use of these pesticides and the black soldier fly in an integrated pest management program in poultry facilities.

The first objective of our study was to examine the effects of two insect growth regulators (IGR), cyromazine and pyriproxifen, on the larval development of the black soldier fly. Our second objective was to determine the susceptibility of wild black soldier fly and house fly populations and Cornell-susceptible house flies to λ-cyhalothrin and permethrin. We compared the susceptibility of the two wild flies to results we recorded for the Cornell-susceptible house flies, which have been in culture for over 20 years.

Materials and Methods

Responses of wild adult house flies and soldier flies to λ-cyhalothrin (Demand® CS, Zeneca, Wilmington, Delaware) and permethrin (Gardstar® 40% EC, Y-Tex, Cody, Wyoming) were determined. Wild adult house flies were exposed to 12 λ-cyhalothrin rates ranging from 0.14 to 12.0 μg/cm^2, and 8 permethrin (Gardstar® 40% EC, Y-Tex, Cody, Wyoming) rates ranging from 6.25 to 800.0 μg/cm^2 with successive rates being diluted with acetone by half. We also exposed flies from a Cornell-susceptible colony to 12 λ-cyhalothrin rates ranging from 0.057 to 5.0 μg/cm^2, and 11 permethrin rates ranging from 1.18 to 11.0 μg/cm^2 with successive rates being diluted with acetone by two-thirds.

Adult soldier flies from a wild colony were exposed to 9 λ-cyhalothrin rates ranging from 0.061 to 1.5625 μg/cm^2 and 11 permethrin rates from 0.65 to 37.5 μg/cm^2. Successive λ-cyhalothrin rates were diluted with acetone by half, while those for permethrin were diluted by one-third each step. Acetone was used as an untreated check in the bioassays for both pyrethroids.

Larval and adult flies used in the experiments were from colonies maintained at the Coastal Plain Experiment Station, Tifton, Georgia. Black soldier fly larvae used in the IGR experiments were from a colony initiated from flies collected in June 1999 from a poultry facility in Alma, Georgia. Wild soldier flies and house flies used in the pyrethroid experiments were from colonies originating from black soldier fly prepupae collected in December 1999 and adult house flies in March 2000 from populations established in a poultry facility located in Hoboken, Georgia. The susceptible house flies were from a colony originating from the Cornell-susceptible strain (Tomita et al. 1995).

Responses of black soldier fly larvae exposed to 9 rates of cyromazine (Larvadex® liquid, Norvartis, Greensboro, North Carolina) ranging from 0.0585 to 1.5 ppm and 8 rates of pyriproxifen (Archer® liquid, Zeneca, Wilmington, Deleware) ranging from 0.0316 to 4 and 6 rates from 58 to 1857 ppm were determined.

IGRs were tested using the following procedures. To achieve a hierarchy of doses, successive pyriproxifen rates were halved in water, while successive cyromazine rates were diluted one-third in water. Water was applied as the control. Replicates of each rate consisted of 35 soldier fly larvae (5 d old) placed in a 454 ml Sweetheart® plastic container (Sweetheart Cup Company, Chicago, Illinois) containing 180 g CSMA (Chemical Specialties Manufacturers Association, Ralston Purina, St. Louis, MO) house

fly larval media treated with 30 ml of the appropriate IGR concentration. Cups containing treated diet and soldier fly larvae were covered with a paper towel and placed in a rearing room (27 ^{o}C, 60-70% RH, and 14:10 light:dark cycle).

Percent larvae failing to reach the prepupal stage and the weights of those that became prepupae were recorded 38 d after initiation of the experiment. Prepupae were identified by change in pigment color from white to black (May 1961). Weights were recorded on a Mettler AT261 DeltaRange® balance. Percent of larvae failing to reach the adult stage for each treatment was recorded 68 d after initiation of the experiment. The experiment was replicated on three occasions from January through August 2000.

Methods used to examine the pyrethroids were adapted from Sheppard and Hinkle (1987). Pesticides were applied in 1 ml of an acetone solution per filter paper, which measured 9 cm in diameter. Treated papers were allowed to air-dry for 24 hr. Papers with the same dose and pesticide were wrapped in aluminum foil and stored at room temperature until their use. Each pesticide rate was represented three times in each bioassay. Adult black soldier flies < 24 hrs in age were used in two bioassays and adults 1 to 12 d in age were used in the final bioassay. Results for experiments conducted with black soldier flies of different ages were compared and determined not to be significantly different. House flies < 6 d old were used in each experiment. Fifteen to 20 of the appropriate fly species were placed in each petri plate on a treated paper and mortality recorded 2 hr later. Mortality was defined as the inability of a fly to walk or remain on its tarsi.

PROBIT analysis was used to analyze the data to determine dosage-mortality regression equations for black soldier flies and house flies (Daum 1970, Russell et al.

1977). Percent data were arcsine transformed prior to being analyzed with Procedure GLM (SAS Institute 1992). Least Significant Difference test (SAS Institute 1992) was used following a significant F test ($P < 0.05$) to separate means per treatment.

Results and Discussion

Cyromazine LC_{50}s for black soldier fly larvae failing to reach the prepupal stage ranged from 0.25 to 0.28 ppm with dosage-mortality regression slopes between 8.14 and 12.04 (Table 7-1). Percent larvae failing to reach the prepupal stage ranged from 8.6% in the 0.0585 ppm rate to 100% in the 1.5 ppm rate. Mean prepupal weight (Figure 7-1) ranged from 188.4 mg per prepupa in the control to 91.6 mg (51% < control) for those reared on diet containing 0.296 ppm cyromazine. LC_{50}s for larvae failing to reach the adult stage ranged from 0.13 to 0.19 ppm with dosage-mortality regression slopes ranging from 3.94 to 7.69 (Table 7-1). Percent larvae failing to reach the adult stage ranged from 70.8% in the 0.078 ppm treatment to 100% in treatments $\geq$ 0.296 ppm.

The current cyromazine rate recommended to suppress house flies in poultry facilities is 5 ppm in the animal feed. Resultant manure has a concentration of 3.3 ppm. This is greater than the LC_{50} range determined for the black soldier fly and would result in the elimination of soldier fly populations and associated benefits. In order to use cyromazine to suppress house flies without reducing black soldier fly numbers, we suggest that the label spray rate of approximately 1000 ppm be used as a spot treatment in areas with dense house fly larvae, while avoiding areas where soldier fly larvae are present.

Significant dosage-mortality regressions for larvae fed pyriproxifen-treated diet could not be determined since mortality at the highest rate (1857 ppm) was no greater

than 45.3%. Mean prepupal weight (Figure 7-2) ranged from 169.3 mg in the control to 236.2 mg (40% > control) for larvae fed diet containing 58 ppm pyriproxifen. Prepupal weight decreased to 124.4 mg (27% < control) for larvae fed diet containing 1857 ppm. For larvae fed diet treated with $\geq$ 116 ppm, percent larvae reaching the prepupal stage in 38 d was 16.7% lower than that recorded for the control, which is due to pyriproxifen delaying pupation. El-Gazzar et al. (1986) recorded prolonged development for flea (Siphonaptera: Pulicidae) larvae exposed to pyriproxifen. We determined that EC_{50}s for percent larvae failing to reach the adult stage ranged from 0.10 to 0.12 ppm with dosage-mortality regression slopes between 1.67 and 2.32 (Table 6-2).

We hypothesize that using pyriproxifen in poultry facilities colonized by the black soldier fly may prolong the larval stage, which could result in greater manure consumption per soldier fly larva. However, reduced adult emergence and possible sterility of those individuals that emerge (Kahru and Anderson 2000) would reduce the black soldier fly population.

Table 7-2 includes estimated LC_{50}s and slopes of regression lines ($\pm$ SE) for the wild black soldier flies and house flies and the Cornell-susceptible house flies exposed to each pyrethroid. LC_{50}s for the black soldier fly ranged from 0.314 to 0.320 $\mu g/cm^2$ for λ-cyhalothrin and 6.46 to 8.43 $\mu g/cm^2$ for permethrin. LC_{50}s recorded for wild house flies ranged from 2.12 to 3.34 $\mu g/cm^2$ for λ-cyhalothrin and 94.24 to 184.23 $\mu g/cm^2$ for permethrin. LC_{50}s for Cornell-susceptible house flies ranged from 0.18 to 0.22 $\mu g/cm^2$ for λ-cyhalothrin and 3.96 to 4.17 $\mu g/cm^2$ for permethrin. These results demonstrate that the wild house flies were 12 to 32 fold more resistant to λ-cyhalothrin and permethrin

respectively, while the black soldier flies responded to doses similar to those effective on Cornell-susceptible house flies.

Permethrin and λ-cyhalothrin are used to treat sites in livestock and poultry facilities frequented by adult house flies and not black soldier flies. Our observations indicate that male adults account for < 3% of the adult soldier flies collected in poultry facilities, while those remaining are individuals ovipositing in the manure (Tomberlin and Sheppard, accepted for publication), which is not treated with the pyrethroids. However, care should be taken to avoid treating potential black soldier fly oviposition sites with these pesticides when colonization and associated benefits are desired.

Acknowledgments

We would like to thank J. Ruberson, W. Berisford, J. Butler and R. Noblet for their helpful comments on this manuscript.

References Cited

Axtell, R. C. and T. D. Edwards. 1970. *Hermetia illucens* control in poultry manure by larviciding. J. Econ. Entomol. 63: 1786-1787.

Axtell, R. C. and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annu. Rev. Entomol. 35: 101-126.

Daum, R. J. 1970. Revision of two computer programs for probit analysis. Bull. Entomol. Soc. Amer. 16: 10-14.

El-Gazzar, L. M., P. G. Koehler, R. S. Patterson, and J. Milio. 1986. Insect growth regulators: Mode of action on the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 28: 424-654.

James, M. T. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bull. Brooklyn Entomol. Soc. 30: 165-170.

James, M. T. 1947. The flies that cause myiasis in man. Misc. Publ. US Dept. Agric. 631: 146-148.

Karhu, R. R. and S. H. Anderson. 2000. Effects of pyriproxifen spray, powder, and oral bait treatments on the relative abundance of nontarget arthropods of black-tailed prairie dog (Rodentia: Sciuridae) towns. J. Med. Entomol. 37: 612-618.

May, B. M. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). N. Z. J. Sci. 4: 55-65.

Meola, R., K. Meier, S. Dean, and G. Bhaskaran. 2000. Effect of pyriproxifen in the blood diet of cat fleas on adult survival, egg viability, and larval development. J. Med. Entomol. 37: 503-506.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: A new computer program for probit analysis. Bull. Entomol. Soc. Am. 209-213.

SAS Institute. 1992. SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute, Cary, NC.

Sheppard, D. C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ. Entomol. 12: 1439 1442.

Sheppard, D. C. and N. C. Hinkle. 1987. A field procedure using disposable materials to evaluate horn fly insecticide resistance. J. Agric. Entomol. 4: 87-89.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value added manure management system using the black soldier fly. Bio. Tech. 50: 275-279.

Tomberlin J. K. and D. C. Sheppard. Accepted 2001. Lekking behavior of the black soldier fly. Florida Entomol.

Tomita, T., N. Liu, F. F. Smith, P. Sridhar and J. G. Scott. 1995. Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroids resistance in the house fly, *Musca domestica*. Insect Molec. Biol. 4: 135-140.

Van Driesche, R. G. and T. S. Bellows, Jr. 1996. Biological Control. Chapman & Hall, New York, New York.

Table 6-1. Regression line slopes ± SE[1] and LC_{50}s (95% confidence interval) ppm for black soldier fly larvae ($n^2 = 3$) failing to reach the prepupal and adult stages when fed CSMA diet treated with cyromazine rates ranging from 0.0585 to 1.5 ppm or pyriproxifen rates ranging from 0.0316 to 1857 ppm

Cyromazine treated diet		Pyriproxifen treated diet	
Failure to reach prepupal stage			
Regression slope	LC_{50}	Regression slope	LC_{50}
12.04 (1.44)	0.26 (0.24-0.27)	*	*
8.14 (0.73)	0.25 (0.24-0.26)	*	*
5.79 (1.11)	0.28 (0.23-0.35)	*	*
Failure to reach adult stage			
7.69 (1.89)	0.19 (0.16-0.23)	2.32 (1.00)	0.12 (0.06-0.17)
3.94 (0.74)	0.13 (0.09-0.16)	1.73 (0.50)	0.12 (0.05-0.47)
4.17 (1.00)	0.18 (0.14-0.21)	1.67 (0.21)	0.10 (0.07-0.13)

[1] Significance set at $P < 0.05$; [2] sample size; * larval mortality level per treatment not significant

Table 6-2. Resistance factors, LC_{50}s and slopes of dosage-mortality regressions for wild black soldier flies, *Hermetia illucens* (L.), and house flies, *Musca domestica* L., exposed to filter papers treated with either λ-cyhalothrin or permethrin in comparison to results generated for susceptible house flies

Hermetia illucens (L.)			*Musca domestica* L.				
Field Population			Field Population			Cornell Colony (Susceptible)	
LC50s (95% CL) µg/cm^2	Slope (SE)	Resistance[a] ratio	LC50s (95% CL) µg/cm^2	Slope (SE)	Resistance ratio	LC50s (95% CL) µg/cm^2	Slope (SE)
			λ-cyhalothrin				
0.34 (0.38-0.31)	5.29 (0.53)		2.12 (2.46-1.85)	2.89 (0.28)		0.18 (0.20-0.16)	5.04 (0.51)
0.31 (0.34-0.29)	5.76 (0.63)		3.34 (4.62-2.57)	2.58 (0.36)		0.22 (0.24-0.19)	4.84 (0.54)
0.32 (0.35-0.29)	5.34 (0.53)		2.26 (2.63-1.96)	2.89 (0.26)		0.21 (0.30-0.16)	4.65 (0.90)
mean = 0.32		1.60	2.56		12.80	0.20	
			permethrin				
8.43 (9.65-7.35)	4.01 (0.49)		184.23 (405.71-104.82)	4.88 (1.19)		4.17 (5.34-3.24)	8.12 (1.98)
6.75 (7.73-5.88)	3.71 (0.38)		94.24 (110.98-79.84)	2.88 (0.27)		4.00 (4.32-3.70)	7.95 (0.99)
6.46 (7.46-5.56)	3.36 (0.36)		108.24 (129.06-90.66)	2.68 (0.24)		3.96 (4.28-3.67)	8.18 (0.96)
mean = 7.21		1.78	128.9		31.91	4.04	

[a]Resistance ratio = mean field black soldier fly or house fly population LC_{50}/mean Cornell house fly colony LC_{50} .

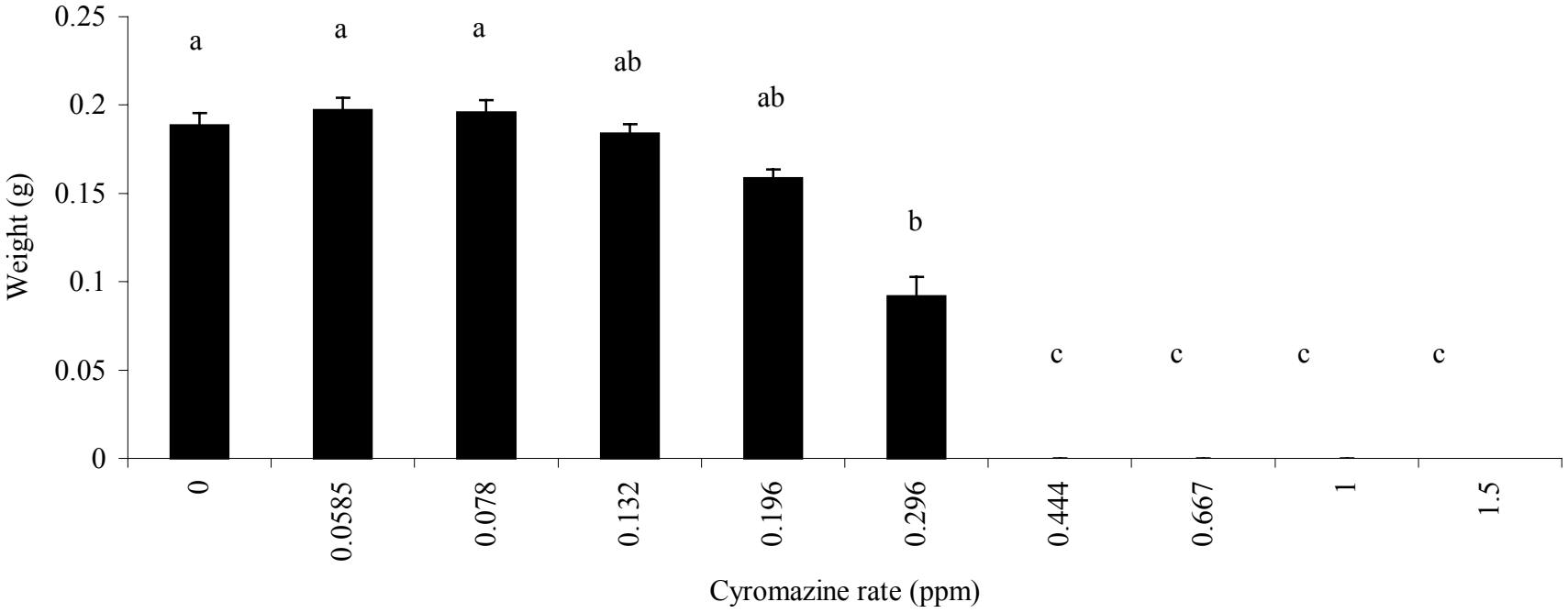

Figure 6-1. Mean prepupal weight (g) ± SE for black soldier fly larvae feeding on diet treated with differing cyromazine rates (ppm). Measurements recorded 38 d after initiation of the experiment. Treatments with different lower case letters were significantly different ($P < 0.5$, LSD).

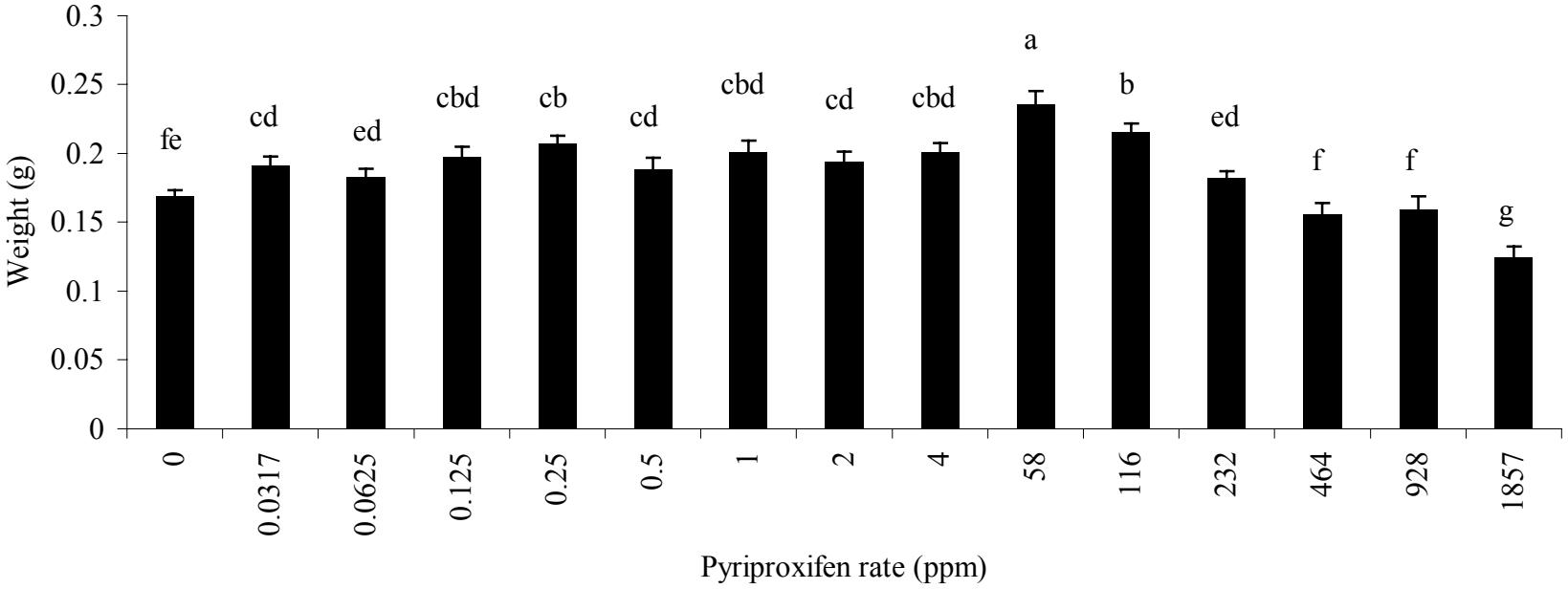

Figure 6-2. Mean prepupal weight (g) ± SE for black soldier fly larvae feeding on diet treated with differing pyriproxifen rates (ppm). Measurements recorded 38 d after initiation of the experiment. Treatments with different lower case letters were significantly different ($P < 0.5$, LSD).

CHAPTER VII

CONCLUSION

Prior to my research, information on the black soldier fly was limited to benefits associated with its colonization of manure in poultry facilities. Larvae in poultry manure could suppress a house fly population from 94 to 100%, reduce manure accumulation 25% annually, and the prepupae could be self-harvested for use as livestock feed. However, very little information was known about the adult biology, or the effects of pesticides used in poultry facilities on larvae and adults of the black soldier fly.

Differences in pre-imaginal development and selected adult life-history traits were determined for black soldier flies reared on three diets. Prepupal and adult characteristics examined for individuals reared on the diets were compared to field-collected prepupae and corresponding adults. Time from egg to adult emergence for black soldier flies reared on the diets ranged from 40 to 43 d at 27 ^{o}C with the larval stage lasting 22 to 24 d. I recorded > 95% larval survivorship to the prepupal stage and 21 to 27% emergence of adults. Adults generally mated 2 d after emergence and laid eggs 2 d after mating.

The life-history data I recorded for black soldier fly development in the laboratory indicated that the diets used, or my methods, are sub-optimal. Adult emergence for the diet treatments was 60 to 70% lower than that recorded for field-collected prepupae. Additionally, prepupae and corresponding adults reared in the laboratory were not as large as the field-collected specimens and had less energy (fat) reserves and produced fewer eggs. Research on enhancing larval media and methods used to rear black soldier flies in the laboratory is warranted. Results from such research could be used to optimize laboratory production of black soldier flies for use as a biological control and waste management agent in poultry facilities.

I conducted a series of experiments examining the attraction of gravid house flies to resources with and without black soldier fly larvae. The initial series of experiments were conducted with a sophisticated olfactometer at the University of Florida in Gainesville, Florida. However, because of the level of variability in these tests, I modified and redesigned my treatments and experiment three times. Three replications were conducted per experiment. Although I did not record significant results for house fly attraction, I did determine that age of the treatments prior to initiating the experiment and the resource used in the treatments influenced house fly attraction. Oviposition bioassays conducted in the laboratory did show that the presence of soldier fly larvae inhibited house fly oviposition in poultry manure. Isolation of larvae did not significantly increase oviposition, indicating that olfaction is most likely involved.

The criteria for attraction were different in the olfactometer experiments than in the oviposition bioassays. Movement towards the odor plume would elicit a positive response in the olfactometer experiment. This movement may or may not have been for the purpose of oviposition in the medium producing the odor. In the oviposition bioassay, immatures resulting from oviposition were counted. This gives a much more definitive answer to the question of acceptance of any given treatment as an oviposition medium.

Future studies that examine the attraction of ovipositing house flies to media with black soldier fly larvae need to take into account media type and age when designing the experiments. Additionally, research should use resources that are known to be colonized by the black soldier fly and the house fly in the wild, such as manure and not artificial

media. An additional research arena that needs to be investigated is the tactile response of the house fly to media inoculated with or without soldier fly larvae.

I examined the effects of pyriproxifen and cyromazine, which are insect growth regulators, on black soldier fly larval development. I determined that black soldier fly larvae, in comparison to house fly larvae, are highly sensitive to these chemicals. Current rates of these chemicals suggested for controlling house flies would also arrest black soldier fly larval development, which would eliminate associated benefits. Therefore, these chemicals should be used carefully or in cases where the black soldier fly and its associated benefits are desired.

I examined the susceptibility of adult black soldier flies and house flies to λ-cyhalothrin and permethrin, which are used to kill adult house flies in poultry facilities. I determined that black soldier flies, unlike the wild house flies, are highly sensitive to these chemicals. Because of this level of sensitivity, poultry producers wanting to reap benefits associated with black soldier flies should apply these chemicals on sites frequented by adult house flies, while avoiding black soldier fly oviposition sites.

Little biological and toxicological information was known about black soldier fly biology prior to my research. Now, a foundation of information has been laid, which will aid in integrating the black soldier fly into house fly control and manure management strategies in poultry facilities. Additionally, information is now available for mass-producing the black soldier fly in the laboratory for possible distribution as a biological control and waste management agent in poultry facilities.